THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

PAGE

EFFECT OF GENERAL EXCITEMENT AND OF FIGHTING ON SOME DUCTLESS GLANDS OF MALE ALBINO RATS. *Toshio Uno* 203

STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS. XI. FURTHER EVIDENCE FOR REFLEX AND ASPHYXIAL SECRETION OF ADRENIN. *W. B. Cannon and R. Carrasco-Formiguera* 215

THE "ALL-OR-NONE" PRINCIPLE APPLIED TO MAMMALIAN NERVES AND REFLEX-ARCS. *J. M. D. Olmsted and W. P. Warner* 228

STUDIES ON ALKALIGENESIS IN TISSUES. II. AMMONIA PRODUCTION IN MUSCLE DURING CONTRACTION. *Olive Pearl Lee and Shiro Tashiro* 244

THE PRODUCTION OF ADRENAL DISCHARGE BY PIQÛRE. *R. Carrasco-Formiguera* 254

THE ACTION OF ULTRA-VIOLET RAYS ON ARBACIA EGGS, ESPECIALLY AS AFFECTING THE RESPONSE TO HYPERTONIC SEA-WATER. *Ralph S. Lillie and Margaret L. Baskervill* 272

THE INFLUENCE OF HYDROGEN SULPHIDE UPON RESPIRATION. *Howard W. Haggard and Yandell Henderson* 289

THE EFFECTS OF PROSTATE SUBSTANCE ON THE METAMORPHOSIS OF THE INTESTINE OF FROG TADPOLES. *Robert W. Hegner* 298

STUDIES UPON THE MECHANISM OF THE INCREASED METABOLISM IN HYPERTHYROIDISM. *Joseph C. Aub, Elizabeth M. Bright and Joseph Uridil* 300

THE RELATIVE GROWTH OF INDIVIDUAL BOSTON SCHOOL BOYS. *W. T. Porter* 311

THE EFFECT OF ADRENALECTOMY UPON THE TOTAL METABOLISM OF THE CAT. *Joseph C. Aub, Jonathan Forman and Elizabeth M. Bright* 326

THE METABOLIC EFFECT OF ADRENALECTOMY UPON THE URETHANIZED CAT. *Joseph C. Aub, Elizabeth M. Bright and Jonathan Forman* 349

PRELIMINARY DILATATION A PHASE OF THE PUPILLARY LIGHT REFLEX. *Joseph Byrne* ... 369

CALCIUM PHOSPHATE METABOLISM IN THE DIAGNOSIS OF RICKETS. *J. F. McClendon* 373

THE ALKALINE RESERVE OF THE BLOOD OF FISH IN RELATION TO THE ENVIRONMENT. *Edwin B. Powers* 380

VOL. LXI—No. 2

Issued July 1, 1922

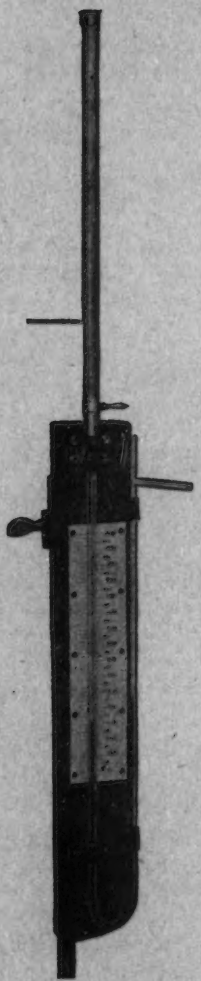
BALTIMORE, U. S. A.

1922

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 5, 1918

Made in United States of America

STUDENT MANOMETER



Price, $11.00

CATALOGUE ON REQUEST

This mercury manometer is constructed of carefully selected annealed glass tubing mounted on a block of hard wood. The horsehair and weight of the usual type of manometer are replaced by a slotted brass tube which serves as a guide for the writing needle. The writing point is brought into contact with the paper by rotating the brass guide tube and is maintained in light but steady approximation by a simple spring mechanism. The scale is not fixed as in other types of manometer, but can be adjusted at "blood pressure zero" for each experiment.

JOSEPH BECKER College of Physicians and Surgeons
437 West 59th Street, NEW YORK

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 61 JULY 1, 1922 No. 2

EFFECT OF GENERAL EXCITEMENT AND OF FIGHTING ON SOME DUCTLESS GLANDS OF MALE ALBINO RATS

TOSHIO UNO

From The Wistar Institute of Anatomy and Biology

Received for publication April 3, 1922

Following great emotional disturbances, both in man and animals, various bodily changes have been noted, such as sweating, the stoppage of saliva, rapid heart beat, trembling, etc. The researches of Pavlov (17) showing physiological changes in the alimentary tract after even insignificant emotional stimulation are familiar. More recently Hatai and Hammett (11) have found that in rats which have been slightly excited by simple rough handling the intestines show a modified reaction to sodium carbonate solution. The investigations of Cannon (1) and his colleagues add to the list of bodily changes, some of which occur in connection with the suprarenal glands. A few years ago Doctor Komine (15) at The Wistar Institute demonstrated an increase of the nitrogenous metabolites in the brains of rats which had been induced to fight for several hours. All these findings indicate that bodily alterations may occur as a result of excitement. In order to investigate further the extent of these changes, I have examined some of the ductless glands from excited animals.

The glands used were the hypophysis, suprarenals and thyroid, and the experiments were planned to determine whether in the male albino rat general excitement and fighting causes changes in the weight, the water content or the action of the extracts, of these several glands.

Material and methods. Forty-one pairs of male albino rats were used. The two animals in each pair were from the same litter, and all were from The Wistar Institute colony and fed with the standard diet. They

ranged in age from 90 to 300 days, but the majority were about 170 days old. All the rats were allowed 3 days or more in the laboratory cage to become accustomed to their new surroundings before the tests were made.

The general stimulation and the fighting were carried out in a box with open wire sides, a little less than one cubic foot in capacity. The wooden bottom of this box was closely studded with the blunt ends of nails rising just above the surface. The nails were connected by a copper wire and these wires in turn with three or four dry batteries. It was possible by suitable arrangements to send a current through the nails in the floor of the box and thus stimulate the rat through its feet. The stimulus was usually given for 4 seconds during each minute throughout the period of the experiment.

The test rat was placed in the box with another pugnacious male and the electrical stimulus applied. This sometimes led at once to the assumption of fighting posture—like that of a boxer—which might be followed by a biting attack. In most cases however the rats do not fight on simple electrical stimulation, but in these instances pricking or pinching the tail of one animal usually serves to start the fight. A few animals did not fight during the entire period of the experiment.

The periods for the tests were 1, 3 and 6 hours, and rats which responded actively were often clearly exhausted at the end of the longer periods. At the close of the experiment the test rat and its control from the same litter, but which had not been stimulated, were etherized, weighed and measured, and the glands dissected out. Each gland was weighed fresh in a closed glass vial and those to be used for determining the percentage of water were then dried at 95°C. for one week. Others were used for the extracts. The method for obtaining these will be given later.

On the weight of the glands and the percentage of water in them. In the accompanying condensed table the mean values for the weight and water data for the 41 pairs examined are tabulated in three groups made on the basis of the duration of stimulation, but these groups have been entered in the order of *increasing body weight.* It should be stated that the mean body weights in the test series were in each instance slightly heavier than those for the controls. These latter are the starred values under the body weight values in the table. This difference is the outcome of choosing the heavier animal for the test.

To make a comparison of the weights of these glands in the test animals with those in the controls, it is necessary therefore to make

a correction for body weight, as all these glands normally increase with an increase in body weight. This is done by determining from the tables in *The Rat* (6), the percentage increase in the weight of each gland on passing from the body weight of controls to that of the test rats. The observed values were than raised by these percentages and the values given in the table are the values thus corrected for body weight.

TABLE 1

The weights and water content of the hypophyses, suprarenals and thyroid glands of stimulated and fighting albino rats and their controls

NUMBER OF PAIRS	AGE IN DAYS	BODY WEIGHT	PERIOD OF STIMULATION AND FIGHTING	HYPOPHYSIS				THYROID				SUPRARENALS			
				Weight		Water per cent		Weight		Water per cent		Weight		Water per cent	
				Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
		grams	*hours*	*mgm.*	*mgm.*			*mgm.*	*mgm.*			*mgm.*	*mgm.*		
10	148	162 *(152)	1	5.79	5.74	75.6	75.3	19.3	19.5	76.7	76.8	23.5	24.1	68.2	68.7
10	170	171 *(160)	6	5.56	5.85	76.1	72.4	22.1	22.0	76.8	77.3	26.4	26.7	68.8	68.4
15	185	201 *(186)	3	6.48	6.84	70.3	73.7	24.4	25.2	76.8	75.6	29.2	28.1	67.7	69.7
Averages				5.94	6.14	74.0	73.8	21.9	22.2	76.8	76.6	26.4	26.3	68.2	68.9
Percentage difference.....				+3.3 per cent				+1.5 per cent				−0.4 per cent			

* Observed body weights of controls.

It seems probable that a corresponding correction should be made also for the percentage of water, but at the moment there are no standard tables which can be used for this purpose. It may be noted, however, that if we may judge from the changes in the percentage of water in the brain, the change would be less than 0.1 per cent for the greatest difference in body weight here presented.

On examining the controls in the table, it appears that the corrected weights for each of the three glands are greater in the oldest and heaviest

group than in the youngest and lightest group, while the weights in the intermediate group tend to be intermediate also. These relations merely serve to support the general correctness of the determinations for the controls and by inference those for the test rats too. Moreover the amount of difference according to body weight is that to be expected from the standard tables (6).

Touching the determinations of the percentage of water in these glands, it is necessary at the outset to admit that, although the greatest care in weighing was used, yet the chances of error were large, owing to the small absolute weights of the dried residue of the glands. This is especially true in the case of the hypophysis. Owing to these conditions no comparisons will be made of the percentages found in the test series with those found in the controls.

It should be noted in passing, however, that the average percentage of water in the controls is highest in the thyroid, a little lower in the hypophysis, and much lower in the suprarenals, and the same is true for the averages in the test rats also.

Table 1 was prepared mainly to permit us to determine whether stimulation and fighting had altered the weight or the water content of the glands under consideration.

If we take first the averages of the three groups and fix attention on the weights of the glands, it appears that the glands from the test animals stand in the following relations:

	per cent
Hypophysis	+3.3
Thyroid	+1.5
Suprarenals	−0.4

Only in the case of the hypophysis is there a difference which might possibly be significant.

On turning to the data for the several groups, it appears that the greatest difference is recorded in the case of the hypophysis after 3 hours of stimulation, and this amounts to 5.5 per cent. Now the statistical constants (probable error of the mean and coefficient of variability) for the two average values here compared are as follows:

Hypophysis = 3 hours' stimulation

	WEIGHT	P. E. M.	C. V.
	mgm.		*per cent*
Control	6.48	± 0.11	11.2
Test	6.84	± 0.14	11.8

In view of these values it seems just possible that the difference of 5.5 per cent may be significant.

A like argument can be made for the difference between the test and control hypophysis after 6 hours' stimulation.

In the case of the other glands, however, for each group of which the constants have been carefully determined, the probable error of the mean is in each case so large as to make the differences valueless. We conclude therefore that there is some evidence that stimulation for 3 and for 6 hours increases slightly the weight of the hypophysis, but there is no evidence that the weights of the other glands have been modified. For the percentage of water, a comparison is not warranted.

Among the 41 cases there were 14 for which the protocols record a severe fight. These occurred in all three groups, but mainly in the group receiving 3 hours' stimulation. A careful analysis of all these data fails, however, to reveal in the weights of the glands any changes which can be referred to the differences in the severity of the combat.

The final outcome of this examination is, then, that among the three glands studied only the hypophysis shows a response, represented by a slight increase in the weight of the test hypophysis in the rats stimulated and fighting for 3 and for 6 hours.

Action of the glandular extracts on the intestinal strip. Extracts of the glands, prepared as previously described, were made in the following manner.

The glands were ground with fine sand and a 0.25 per cent solution was made with Tyrode's solution, from which sugar was omitted. The emulsion was frequently shaken and left at room temperature for about 2 hours. Both the controls and tests were treated in the same way. In order to test the activity of the glandular extracts, a special glass bottle (Richards and Wood (18)), filled with Tyrode's solution and permitting a supply of oxygen, was put into the water bath which was kept at a temperature of 37°C., and within this the strip of the intestine (doudenal portion) was immersed. The strip was so attached that changes in its length and rhythmic contractions could be recorded. After the normal rhythm had been established, a definite amount of the extract, first from the control gland and second from the test, was added to the chamber and a tracing made to show the effect of each solution which had been added.

As has been abundantly shown by other investigators, the normal responses of the intestinal strip to the extracts of these several glands are as follows:

1. Thyroid extract causes a prompt contraction of the strip.

2. Suprarenal extract causes a marked relaxation of the strip.

3. The hypophyseal extract causes a relaxation of the strip, sometimes preceded by a slight short contraction.

With each of these glands an extended series of experiments was carried out to determine whether the extract from the glands of the test rats caused any change in the response of the intestinal strip.

A study of the accumulated tracings shows that the extracts from the test thyroid and from the test suprarenals affect the intestinal strip just as the corresponding extracts from the controls do. Stimulation and fighting therefore produce no evident changes in these two glands which affect the character of their extracts in their action on the isolated intestinal strip.

In the case of the hypophysis however a difference appears, and we shall limit our further discussion to this case.

We shall present in the first instance the effects of stimulation and fighting combined on the response of the intestine for each of the three periods, citing but one example for each period.

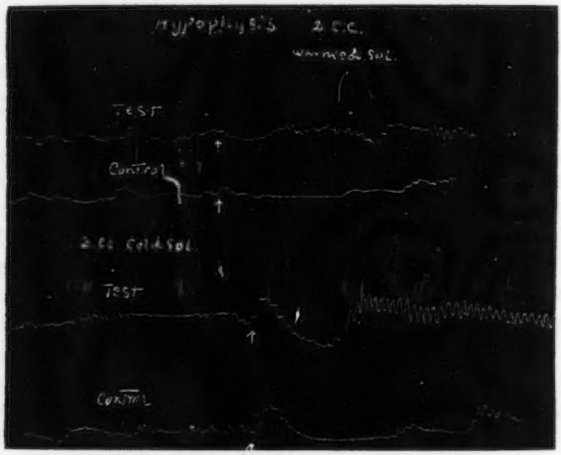


Chart 1. Stimulated and fighting for 1 hour. Fight severe. Age, 104 days.

Stimulated for 1 hour: Altogether the extracts from four males, two for control and two for test were employed. The average age was 104 days; the fight was severe.

Suprarenals: 0.5 cc. of the fresh extract was added. The test and control showed the same relaxation.

Thyroid: 2 cc. were added. The contractions are identical in the test and control.

Hypophysis: 2 cc. were added in both cases. The test showed the contraction of intestine while the control showed *relaxation*. The results are clearer in the second case. Chart 1.

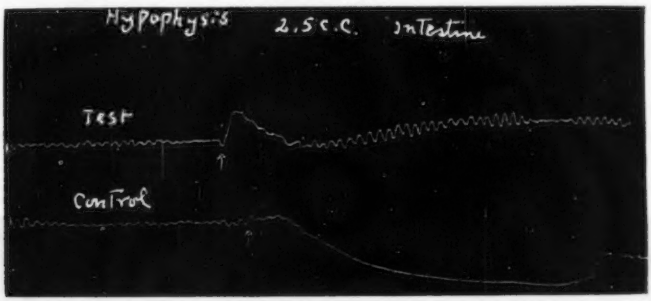


Chart 2. Stimulated and fighting for 3 hours. Fight severe. Age, 262 days.

Stimulated for 3 hours: Altogether extracts from four males, two for control and two for test, were employed. The average age was 262 days. The fight was *severe* and *continuous*.

Suprarenals: 0.5 cc was added. The relaxation is indentical both in test and control.

Thyroid: 2.0 cc. were added. The test extract produced almost the same contraction as the control.

Hypophysis: 2.5 cc. were added. We notice again a contraction in the case of the test hypophysis and a relaxation in the control. Chart 2.

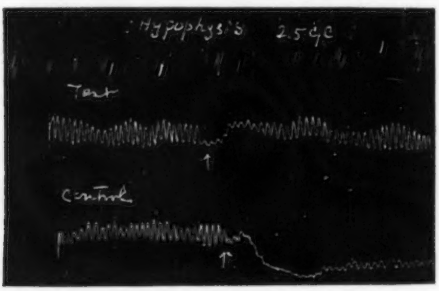


Chart 3. Stimulated and fighting for 6 hours. Fight severe. Age, 201 days.

Stimulated for 6 hours. Altogether the extracts from four males, two for control and two for test, were employed. The average age was 201 days; the fight was severe.

Suprarenals: 0.5 cc. of the fresh extract was added. The relaxation is identical in both test and control.

Thyroid: 2 cc. were added. The contraction is the same in test and control.

Hypophysis: 2.5 cc. were added. The tracing given by the test hypophysis gives very clear reaction showing a *contraction*, while the control showed the *relaxation*. Chart 3.

As already stated, the foregoing observations were made with extracts from test rats which had been stimulated and had also fought. To determine whether either of those forms of excitement taken alone would produce the effect which they gave when combined, further tests were made.

Chart 4. Severe fight for 3 hours without electrical stimulation. Age, 259 days.

The results of 3 hours of fighting without electrical or other stimulation: Two male albino rats of 259 days; *severe fighting.*

Suprarenals: 1 cc. of fresh extract was added. We find no difference between test and control.

Thyroid: 2 cc. were added. The contraction is identical in both test and control.

Hypophysis: 1.7 cc was added. The test rat gave a tracing which showed a *contraction* of the intestine, while the control showed the *relaxation*. Chart 4.

The tracings show that fighting alone for 3 hours can bring about the change in the hypophysis. Shorter periods were not tried. To contrast with the foregoing, we have a test of electrical stimulation for 1 hour in rats which were not allowed to fight.

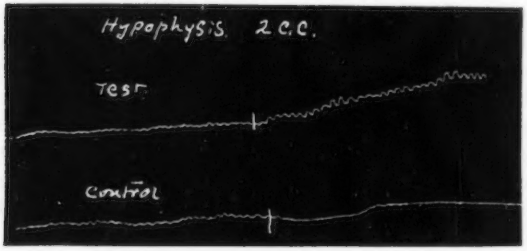


Chart 5. Electrical stimulation alone, for 1 hour. Age, 325 days.

Electrical stimulation alone for 1 hour: Two male albino rats 325 days old were employed. The test rat showed slight excitement as judged from its actions at the moment of the electrical discharge.

Suprarenals: 1 cc. of fresh extract was added. The action of the test and control showed the same degree of relaxation.

Thyroid: 2 cc. were added in each case. The contractions are identical in both test and control.

Hypophysis: 2 cc. were added. The test rat showed *contraction* of the intestine, while the control showed *relaxation*. Chart 5.

When the time of stimulation was made 30 minutes or 10 minutes, the reaction was like that for the normal control gland.

It is worth noting, in connection with the difference in the response to the test extract, that aside from the direction of the tracing, which indicates a contraction of the intestine, the test tracings always show a persistence of the rhythmic contractions, a feature which tends to disappear in the tracings from the extract of the control hypophysis.

The first step in the explanation of these results is given by the fact that when a series of rat hypophyses is divided into the nervous and glandular portions, and the respective extracts from these portions applied to the intestinal strip, the glandular extract causes a marked contraction, while the extracts of the nervous portion give a relaxation, and the two extracts combined give a response which is intermediate. I have made this experiment and Degener (5) has recently reported the same result.

This enables us to state that stimulation or fighting changes the hypophysis so that the extract from the test hypophysis acts on the strip of the intestine like the glandular extract taken alone. How this change comes about is, however, still to be determined.

From the foregoing experiments we conclude therefore that in the male rat stimulation and fighting do not modify the extracts of the thyroid

or the suprarenals when these extracts are applied to the intestinal strip, but they do modify the extract of the hypophysis so that it causes a contraction of the intestinal strip, whereas the normal effect produced by hypophyseal extracts from unexcited rats is a relaxation.

In the rats, both stimulated and fighting, the effect is somewhat more marked after 6 hours of stimulation than after 1 hour. Fighting alone, without electrical stimulation, or electrical stimulation without fighting, give results of the same character; but stimulation alone for 30 minutes or for 10 minutes, did not serve to produce the change.

Discussion. There is an abundant literature on the changes in the weight and iodine content of the thyroid gland under various conditions, both normal and abnormal. There is no doubt therefore that both the weight and condition of this gland are subject to change, and the foregoing results merely show that in the male rat the experimental conditions here used, for from 1 to 6 hours, did not serve to produce an evident change in weight or water content, nor did they alter the effect of the extract of the gland on a strip of the intestine.

The statement just made with regard to the thyroid holds true for the suprarenals also. One may add merely that in view of observations, such as those by Cannon (1), Elliott (7), Crile and Rowland (3), and Stewart and Rogoff (19), it is rather surprising that even the short periods of excitement which I used did not produce some change.

If the results which we have obtained as the effect of 3 to 6 hours stimulation and fighting on the weight and action of the extract of the hypophysis are accepted, then we can conclude that a positive response is made by this gland. For changes in the hypophysis under various conditions, there is much evidence already at hand.

For example, in pregnancy Naegeli (16), in castration Hatai (8), (9), and in inanition Jackson (13), the hypophysis is said to alter not only its size, but structure also. Herring (12) found in rabbits that definite histological changes appeared in it after thyroidectomy. He found also in cats about 1 week after operation that the glandular bodies and hyaline material in the posterior lobe were more plentiful than was normally the case. Degener (4) determined in rabbits which were killed at periods varying 10 to 179 days after thyroidectomy that this gland was distinctly heavier in thyroidectomized animals than in the controls.

Recently Degener (5) has also reported that in rats on an oatmeal and on a vegetable diet, the extract from the hypophysis causes a contraction of the intestinal strip, while the extract from the control

glands gives a relaxation. These observations indicate clearly that the hypophysis responds readily to the physiological changes as well as to the removal of other ductless glands.

Recently Cow (2) attempted to explain the cause of reversed action of adrenalin on the uterus of the pregnant guinea pig. Cow assumes that the hypophyseal hormone may be responsible, since the hypophysis is known to increase in size during pregnancy, and it is conceivable that the reversed action of the adrenalin may be due to intense sensitization as the result of the hyperactivity or increased secretion of the hypophyseal hormone.

CONCLUSIONS

Forty-one male albino rats, stimulated and fighting for from 1 to 6, hours, were examined to determine whether there was a modification in the weight, the water content and the action of the extracts, of the thyroid, suprarenals and hypophysis.

1. The results for the thyroid and the suprarenals were entirely negative.

2. The results for the hypophysis were positive. After 3 to 6 hours' stimulation and fighting, the hypophysis increased in weight, but no significant change in the percentage of water was found. The extracts of the test glands caused a contraction of the intestinal strip—the response characteristic for the glandular portion of the hypophysis—while that of the control glands gave the usual relaxation.

3. This modification of the extract followed stimulation alone or fighting alone—but did not appear until after 1 hour of excitement.

4. The rhythmic contractions of the intestine always persisted under the test extract.

5. The average percentage of water in the controls was for the thyroid 76.8 per cent, the hypophysis 74.0 per cent and the suprarenals 68.2 per cent.

BIBLIOGRAPHY

(1) Cannon: Bodily changes in pain, hunger, fear and rage. New York and London, 1915.
(2) Cow: Journ. Physiol., 1919, lii, 301.
(3) Crile and Rowland: Anoci-association. Philadelphia and London, 1915.
(4) Degener: Quart. Journ. Exper. Physiol., 1913, vi, 111.
(5) Degener: This Journal, 1922, lx, 107.
(6) Donaldson: The rat. Data and reference tables. Memoirs of the Wistar Institute of Anatomy and Biology, no. 6, 1915.
(7) Elliott: Journ. Physiol., 1912, xliv. 374.

(8) HATAI: Journ. Exper. Zoöl., 1913, xv, 297.
(9) HATAI: Amer. Journ. Anat., 1913, xv, 87.
(10) HATAI: Anat. Rec., 1915, ix, 647.
(11) HATAI AND HAMMETT: This Journal, 1920, liii, 312.
(12) HERRING: Jour. Exper. Physiol., 1908, i, 281.
(13) JACKSON: Amer. Jour. Anatomy, 1915, xviii, 75.
(14) JACKSON AND STEWART: Amer. Jour. Dis. Child., 1919, xvii, 329.
(15) KOMINE: Jour. Comp. Neurol., 1919, xxxi, 69.
(16) NAEGELI: Inaug. Diss. Freiburg, i. b. in A. Biedl Innere Sekretion, 2 auf. II teil, 104.
(17) PAVLOV: The work of the digestive glands (trans. by W. H. Thompson), 2nd ed., London 1910.
(18) RICHARDS AND WOOD: Jour. Pharm. Exper. Therap., 1914, vi, 283.
(19) STEWART AND ROGOFF: Jour. Exper. Med., 1916, xxiv, 709.

STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XI. Further Evidence for Reflex and Asphyxial Secretion of Adrenin

W. B. CANNON and R. CARRASCO-FORMIGUERA

From the Laboratories of Physiology in the Harvard Medical School

Received for publication April 15, 1922.

Stewart, Rogoff and Gibson have properly insisted that when any part of an animal is used to test whether a procedure causes increased adrenal secretion, it must be shown that the procedure itself does not influence the part in a confusing manner (1). In a previous paper of this series Cannon and Rapport showed that the rate of the denervated heart is unaffected by afferent stimulation if the adrenal glands are excised and if the hepatic nerves are severed (2).

With the provision just mentioned the facts brought out by Cannon (3) and by Cannon and Rapport (2) attest *a*, that the denervated heart is a satisfactory indicator of adrenal secretion; *b*, that, if all the mesenteric nerves are cut and the renal arteries and the aorta immediately below them are tied, so that the adrenal glands are the only innervated abdominal viscera having a blood supply, the pulse is markedly accelerated when an afferent nerve is stimulated; and *c*, that this response fails if the adrenal glands are removed. Although the results thus secured cannot be explained by "redistribution of blood in the body," and although tests show that the amount of adrenin liberated reflexly is more than ten times the amount of the steady unvarying secretion which has been assumed to be "normal" (2), the emphasis which has been laid on negative results justifies the presenting of still more evidence for reflex and asphyxial stimulation of an adrenal discharge.

Further evidence for reflex secretion of adrenin. There is general agreement among investigators that adrenal secretion is subject to splanchnic impulses. The most discriminating method of demonstrating splanchnic control of the adrenin output was that employed by Stewart, Rogoff and Gibson (1). When they stimulated a splanchnic

nerve, they noted dilatation of the pupil in the eye sensitized by removal of the superior cervical ganglion—an effect typical of adrenin circulating in the blood. They then blocked the return of blood from the adrenal glands during a second stimulation, and, after observing under these circumstances an absence of the former effect, they removed the block and then saw again the typical pupillary response. Furthermore, they found that the time which intervened between the release of the pent blood and the pupillary reaction was approximately the same that intervened between the start of stimulation or the injection of adrenalin into the femoral vein and the reaction. We have undertaken to retrace, so far as possible with reflex stimulation, the steps taken by Stewart, Rogoff and Gibson with direct splanchnic stimulation. In our experiments cats were used under ether or chloralose anesthesia. The hepatic factor was invariably eliminated by section of the hepatic nerves.

When the venous path from the adrenal glands is blocked the reflex response fails. Blocking of the passage of blood away from the adrenal glands into the general circulation is a procedure which, we found, must be carried out with great caution. The most certain way to open as well as to close the cava at a given signal was by use of a long delicate clamp, the slender tips of which were incased in rubber to prevent injury to tissues. This clamp was set astride the cava close below the liver, and the abdominal wall was then fastened shut. By means of the clamp the cava could be closed or opened at will, just above the mouths of the lumbo-adrenal veins. When the cava is thus closed the rate of the denervated heart soon becomes slower. In table 1 are figures showing the heart beats in 5-second periods beginning with a closure of the cava which lasted 45 seconds. The fall in heart rate revealed by these figures cannot be due to the lower blood pressure induced by closing the inferior cava (2). It is doubtless the result of excluding from the circulation the adrenin which is spontaneously secreted under experimental conditions. It is quite similar to the prompt slowing of the pulse seen when the secretion is reflexly inhibited by exciting centrally the vagus trunk (5).

After the inferior cava has been closed as above described, and no stimulation has occurred, removal of the block is followed by a restoration of the previous heart rate, and in some instances by a slightly higher rate for a brief period lasting 10 or 15 seconds. This effect can reasonably be interpreted as due to the release of accumulated adrenin, perhaps a little increased by stimulation of the glands by movement of the

clamp, and its continued secretion thereafter. In the series of observations recorded in table 1, for example, the initial heart rate per minute, the minimal rate from closure of the cava, the maximal rate when the block was released, and the subsequent rate were as shown in table 2.

TABLE 1

Heart beats in nine 5-second periods during which the inferior vena cava was closed above the lumbo-adrenal veins

DATE	TIME	1	2	3	4	5	6	7	8	9
January 24	12:00	16	16	15.7	15.7	15.5	15.5	15.5	15.5	15
January 26	10:50	18	18	17.5	17.5	17.5	17.5	17	17	16.5
January 26	11:26	18	18	17.5	17.5	17.5	17	17	17	16.5
February 7	3:35	15	15	15	14.7	14.7	14.5	14.2	14.2	14.2
February 8	11:57	14	14	14	13.5	13.5	13.5	13.5	13.5	13
Average.....		16.2	16.2	15.9	15.8	15.7	15.6	15.4	15.4	15

TABLE 2

Initial, minimal, maximal and final heart rates per minute, during and after closure of the inferior vena cava above the adrenal veins

DATE	TIME	HEART RATES PER MINUTE			
		Initial	Minimal	Maximal	Final
January 24	12:00	192	180	204	192
January 26	10:51	216	198	216	210
January 26	11:26	216	198	222*	216
February 7	3:35	180	170	192†	178
February 8	11:57	168	156	168	168

* Duration of the increase above the initial rate, 10 seconds.
† Duration of the increase above the initial rate, 15 seconds.

Although it is possible to slow the heart rate by merely closing the cava above the adrenals in the undisturbed animal, it is impossible, in our experience, to do so if an afferent nerve is stimulated during the closure. For example, on January 26, at 11:26, when the cava was closed without nerve stimulation, the heart beats in 5-second intervals during the 45 seconds of closure were reduced as shown in table 1. The gradual fall in these figures, which are repeated below, should be contrasted with the gradual rise in the figures for 11:31 and 11:38,—the

heart beats in the same intervals during the same period of closure, but with brachial stimulation during the first 30 seconds:

11:26	18	18	17.5	17.5	17.5	17	17	17	16.5
11:31	17	17	17.2	17.7	17.7	18.2	18	17.5	17.5
11:38	17	17.5	17.7	17.7	17.7	17.7	18	18	17.5

Stimulation was accompanied by a slight, but actual, increase in cardiac rate though the direct venous return by way of the cava was blocked. If we stimulated during the closure the only way in which we were able to duplicate the result (i.e., the slower pulse) shown in table 1 was by tying the lumbo-adrenal veins lateral to the glands, tying all the neighboring small veins, the renal vessels and the inferior cava immediately below them. To prevent pooling of the blood in the hind limbs the aorta was tied before tying the cava. When all these precautions were taken to avoid any escape of blood from the adrenal veins by devious by-ways into the general circulation, the rate of the denervated heart would fall during closure of the cava although an afferent nerve was being stimulated. This procedure we have followed in our experiments except when otherwise mentioned.

The effect of blocking the passage of blood away from the adrenal glands is shown by the original records in figure 1. At 2:01, 40 seconds after brachial stimulation began, with the cava open, the pulse was increased from 16.2 to 19 beats in 5 seconds, i.e., from 195 to 228 per minute. At 2:08, with the cava completely closed, as described above, the same stimulus applied for the same length of time caused no acceleration whatever—indeed, the heart rate became slower. The record taken at 2:15 shows that the failure at 2:08 was not due to injury of the glands, for with the cava again open, brachial stimulation again increased the rate, from 180 to 200 beats per minute.

In this experiment the only difference between presence and absence of acceleration of the heart when the brachial nerve was stimulated was the difference between freedom and confinement of the blood flow from the adrenal glands. Under analogous conditions, when blocking of the venous path abolished the pupillary response to splanchnic stimulation, Stewart, Rogoff and Gibson used the observation to support the inference that the response was "due solely to the passage of a substance in the blood stream from the adrenals." We do likewise, and account for the faster heart rate by reflex increase of adrenal secretion, just as they account for dilatation of the pupil by an increase due to splanchnic

impulses. That the effect cannot be explained by "redistribution of blood in the body," as Stewart and Rogoff later argued (4), has already been proved (2).

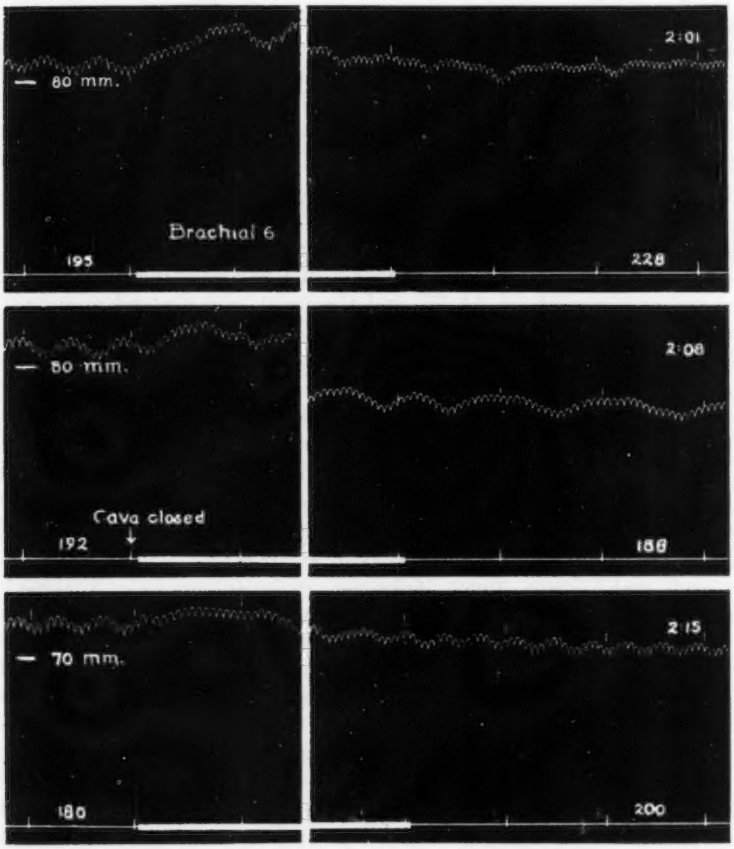


Fig. 1. Beginning and end of records of the beat of the denervated heart, when the right brachial nerve was stimulated for 30 seconds. Time line shows here and in other records, 5 seconds, and zero blood pressure. At 2:01, the stimulation increased the heart rate 33 beats per minute. At 2:08 venous return from the adrenal glands was blocked at the start of stimulation, and the heart rate, instead of increasing, fell 4 beats. At 2:15, with the blood flow free as at 2:01, afferent stimulation again increased the rate, this time by 20 beats per minute.

When the response has been abolished by blocking the venous path, it appears on releasing the block. Figure 2 shows the effect, first, of brachial stimulation for 30 seconds, at 11:51; 45 seconds after stimulation started, the heart rate had risen from 168 to 204, an increase of 36 beats per minute. Next, at 11:57 the cava was closed for 45 seconds; the rate fell from 168 to 156 beats per minute. When the cava was opened the heart rate rose again to its former level, 168 beats per minute. The next procedure combined these first two. At 12:03 the cava was closed and simultaneously stimulation was started and continued for 30 seconds as before. Forty-five seconds after stimulation started, the heart rate, instead of being increased by 36 beats per minute, was decreased by 8 beats—from 164 to 156. Now the clamp was released and the pulse sharply rose from 156 to 180 beats per minute. The increment occurred much more quickly than when the cava was merely closed and opened (cf. 11:57) and the heart rate, instead of merely returning to its former level (164) as it did at 11:57, or showing a momentary increase of 8 or 12 beats as sometimes occurs, rose 16 beats above the former level and remained at the maximum (180) not for 10 or 15 seconds (cf. table 2) but for 30 seconds, and continued for at least 15 seconds longer at 174 beats per minute.

The observations just detailed not only show that the denervated heart fails to respond characteristically to reflex stimulation if the venous path from the adrenals is closed, but promptly does respond in a typical manner when the path is reopened. Again Stewart, Rogoff and Gibson, under analogous circumstances, have used such evidence to justify their conclusion that the characteristic effect on an organ sensitive to adrenin, when the splanchnic nerve is stimulated, is due solely to material delivered into the blood stream by the adrenal glands. And again we do likewise, calling especial attention to the faster rise and the faster rate induced when the brachial nerve was stimulated, as evidence of an extra adrenal discharge thereby evoked.

The time interval between release of a venous block, or injection of adrenalin, and the start of response is approximately the same as for reflex or splanchnic stimulation. The mode of registration used by us to record the beating of the denervated heart does not permit us to state the exact moment when the rate begins to change in consequence of afferent stimulation. All that can be claimed is that the method tells within what period of 5 seconds the acceleration starts. Records of large numbers of tests show that with average blood pressure the faster rate almost invariably starts in the second or third 5-second period

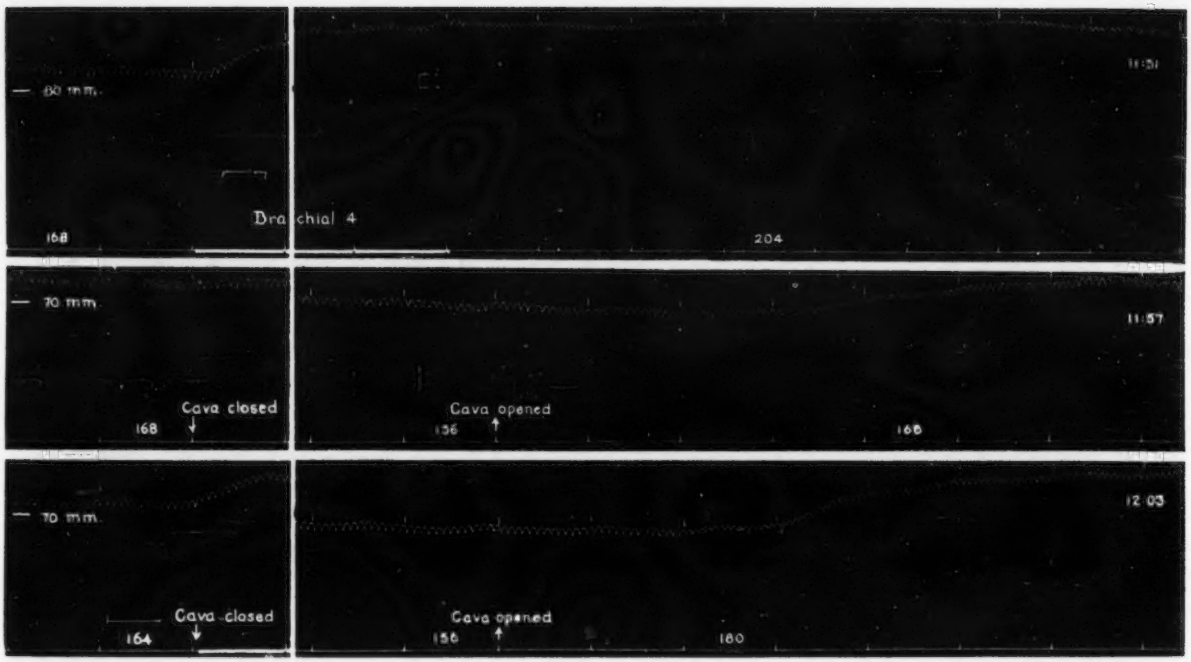


Fig. 2. Beginning and end of records of beat of denervated heart, when the right brachial nerve was stimulated for 30 seconds (at 11:51 and 12:03). At 11:51, the stimulation increased the heart rate 36 beats per minute. At 11:57, the venous return from the adrenal glands was blocked for 45 seconds; the heart rate fell 12 beats, but recovered its former level when the block was removed. At 12:03, brachial stimulation during a second blocking of the flow; the rate fell 8 beats, but quickly rose 24 beats on releasing the block.

after stimulation is started. When the cava and accessory veins are completely tied off, as described above, the delay may be longer than that, probably because there is not a large stream of blood sweeping through the cava and carrying the secreted adrenin rapidly to the heart. For example, when the block was released at 11:57 (fig. 2) it was not until the fourth 5-second period that the faster rate was clearly established. In order to illustrate the fact that the time interval between the start of stimulation and the start of cardiac acceleration is sensibly the same as the time interval between the release of the venous block and the start of acceleration, we present the experiment, illustrated in figure 3, in which the cava was merely blocked above the opening of the lumbo-adrenal veins, without any tying of the venous tributaries below. As already explained, if the brachial is stimulated under these circumstances, there is evidence of escape of adrenin into the general circulation, as shown by failure of the heart rate to fall or the presence of a slight rise. When the blood was flowing freely along the cava, as at 11:22, the acceleration began within 10 seconds after the stimulus was first applied. When the brachial was stimulated again for 30 seconds at 11:31, with the cava blocked, the rate did not fall as it does if the venous byways are all closed (cf. fig. 1, 2:08), but also it did not rise in the typical manner. The rate at the start of stimulation was 204; after stimulation was stopped, and just before the cava was opened again, the rate was 210. Again within 10 seconds after the opening, the rate began to rise and went up to 234 beats per minute.

As already mentioned, Stewart, Rogoff and Gibson compared the time interval between stimulation and the pupillary response, with the time interval between adrenalin injection (into the femoral vein) and the response, and found the two intervals approximately the same. Since the vascular channels were of about the same length, the conclusion was drawn that the dilatation was due to the passage of an adrenal discharge to the eye in the blood stream.

The same evidence is available in relation to reflex stimulation. In table 3 are given the increases of heart beat in 5-second intervals, starting with brachial stimulation in column A, and starting with injection of small amounts of adrenalin in column B. (These cases are reported in more detail in a previous paper (2).) Inspection of the table shows that almost invariably, under either circumstance, the cardiac acceleration begins in the second or third period. The *average* increments of rate are closely parallel in the second and third periods. A plotting of these averages yields a curve the shape of which indicates

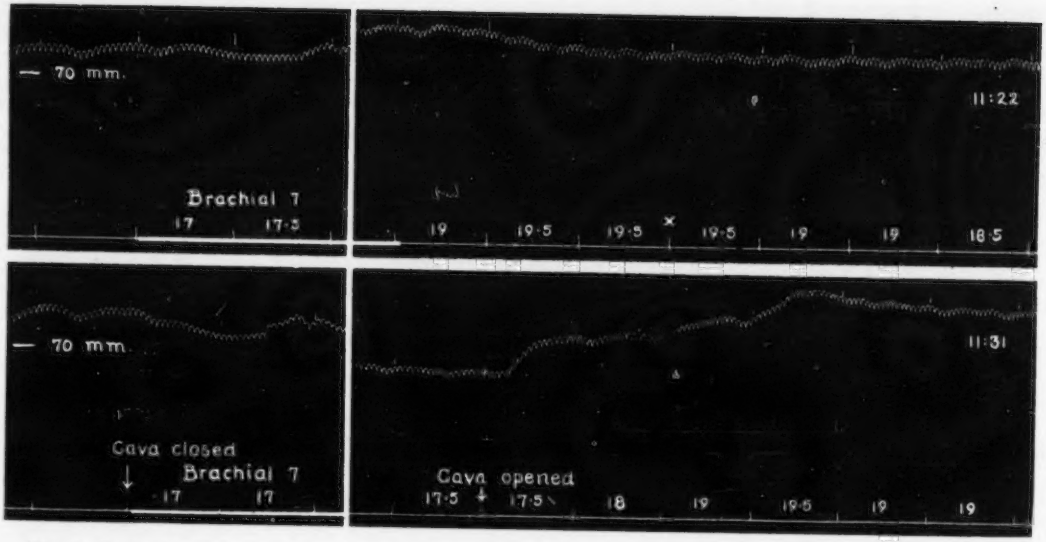


Fig. 3. Beginning and end of records of the beat of the denervated heart, when the right brachial nerve was stimulated for 30 seconds. At 11:22, acceleration of the rate began within 10 seconds after the stimulus was started; at *X*, 45 seconds after the start, the rate was 234 beats per minute. The point *X* corresponds in time to the opening of the cava in the record for 11:31. At 11:31, brachial stimulation (30 seconds) during closure of the inferior cava above the adrenal veins; when the cava was opened, 45 seconds after the start, the rate was 210. Acceleration of the heart began within 10 seconds thereafter and rose to 234 beats per minute.

that the heart may be expected to begin to beat faster about 8 or 9 seconds after the start of stimulation; it does not invariably do so, as is manifest in the cases (in table 1) of no acceleration during the second period.

TABLE 3

Increase of heart beat, in 5-second periods, starting with (A) brachial stimulation, and (B) intravenous injection of adrenalin (1:100,000) at a uniform rate

DATE	PULSE INCREASE IN 5-SECOND PERIODS							
	TIME	(A) Starting with stimulation			TIME	(B) Starting with injection		
		First	Second	Third		First	Second	Third
October 18					12:05	0	1.5	2.5
					12:11	0	0.5	1.5
					12:15	0	0.5	2.5
					12:20	0	0.5	1.5
					12:25	0	0.0	1.0
					12:29	0	0.0	1.0
					12:36	0	0.0	1.5
December 8	12:34	0	0.5	0.5				
December 9	11:01	0	0.0	0.25				
December 10	10:32	0	0.0	0.25	1:06	0	0.0	0.5
	10:44	0	0.5	1.5	1:18	0	0.0	0.5
	10:57	0	0.5	1.5				
	12:14	0	1.0	1.5				
December 11	10:12	0	0.25	1.75	10:43	0	0.0	1.0
	10:23	0	0.0	1.5	10:52	0	0.0	1.0
	11:04	0	0.5	1.0	12:14	0	0.5	1.0
December 13	10:21	0	0.0	0.5	12:18	0	0.0	0.5
	11:18	0	0.25	0.75	12:34	0	0.5	1.5
	12:07	0	0.0	1.0	12:41	0	0.5	1.0
	12:10	0	0.0	1.5				
December 14	10:51	0	0.0	1.0				
December 15	10:24	0	0.5	0.5	11:08	0	0.0	0.0
	10:38	0	0.5	1.0				
	11:04	0	1.0	1.0				
December 16	10:42	0	0.0	1.0	11:15	0	0.0	0.5
					11:24	0	0.0	1.0
Average		0	0.3	1.0		0	0.25	1.1

The foregoing results are concordant with those reported by Stewart, Rogoff and Gibson for splanchnic stimulation. They found that the pupil could begin to dilate in 7 or 8 seconds after starting the stimulus, but in some instances the delay was 12 or 13 seconds or longer. We

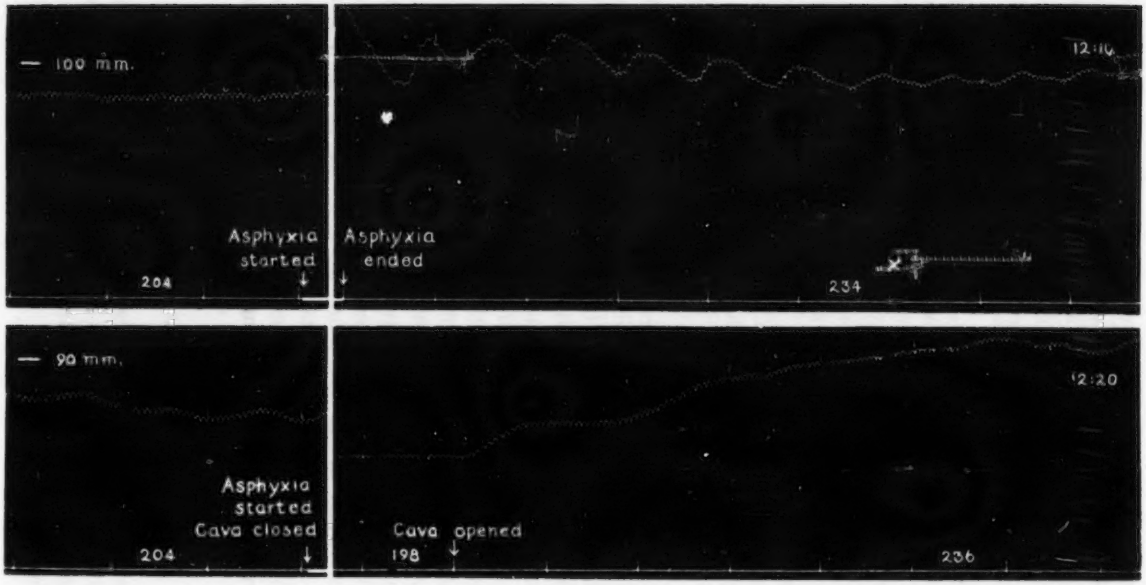


Fig. 4. Beginning and end of records of the beat of the denervated heart. At 12:10, asphyxia for 45 seconds increased the heart rate 30 beats per minute. The point *X* (75 seconds from the start) corresponds in time to the opening of the cava in the record for 12:20. At 12:20 asphyxia for 45 seconds, with the cava closed 75 seconds; the heart rate fell 6 beats while venous flow was blocked, but rapidly rose 38 beats when the block was removed.

have found a similar latent period on exciting the splanchnics when we recorded the beat of the denervated heart. In a series of instances the average increments in the first, second and third 5-second periods were respectively 0, 0.5 and 1.5 beats—again yielding a curve which places the start of acceleration at about 8 seconds after the stimulus is first applied.

FURTHER EVIDENCE FOR ASPHYXIAL SECRETION OF ADRENIN. In two previous communications (6), (3) experiments have been described that show the effects of asphyxia on adrenal secretion. In a preceding paper on adrenal control (2) the testimony was restricted to reflex effects on the glands, but solely for purposes of clarifying the issue. Asphyxia will bring the sympathetic system into action quite as effectively as strong excitation of an afferent nerve, and it should, therefore, induce activity in the adrenal medulla. The method of blocking the venous pathway can be used to show that the faster rate of the denervated heart associated with asphyxia is dependent on extra adrenal discharge.

In the experiment illustrated in figure 4 the heart was denervated, the hepatic nerves were cut and the venous tributaries to the inferior cava and to the adrenals completely tied off in the manner already described. As the figure shows, asphyxia produced by closing the tracheal cannula for 45 seconds increased the heart rate, 70 seconds after the start, from 204 to 234 beats per minute (at *X*). The asphyxia was then repeated for the same length of time, but with the cava clamped. The cava was not opened for 30 seconds after the asphyxia period terminated. Seventy seconds after the start the heart rate, instead of being faster, was actually reduced from 204 to 198, just as in other experiments with the cava closed (cf. figs. 1 and 2). The clamp was then released and within 15 seconds the rate was rising; it continued to rise until it reached 236 beats per minute. In other words, here is evidence of the same character as that previously given for reflex secretion of adrenin, demonstrating that there is also an asphyxial secretion.

SUMMARY

After severance of the hepatic nerves the acceleration of the denervated heart when an afferent nerve is stimulated is due solely to the passage of adrenin from the adrenal glands to the heart. The evidence for this statement is as follows:

1. If the venous path from the adrenal glands is blocked in such manner that the only change is a shutting of adrenal blood out of the circulation, the reflex response, which previously occurred, is prevented (see figs. 1 and 2).

2. After removal of the venous block the reflex response can again be obtained (see fig. 1), and the released adrenal blood causes a greater cardiac acceleration than if, during the block, an afferent nerve has not been stimulated (see fig. 2).

3. If the free flow through the inferior cava is stopped above the opening of the lumbo-adrenal veins, the typical reflex response fails; the time interval between release of the pent blood and the effect on the heart is approximately the same as that between the start of afferent stimulation and the effect on the heart (see fig. 3).

4. The time interval between the start of reflex stimulation and the beginning of the cardiac response is approximately the same as that seen after exciting a splanchnic nerve or injecting a physiological amount of adrenin into a femoral vein (see table 3).

Asphyxia for 45 seconds induces a more rapid beat of the denervated heart. This effect fails if the passage of blood away from the adrenal glands is prevented, and appears when the blood is released.

BIBLIOGRAPHY

(1) Stewart, Rogoff and Gibson: Journ. Pharm. Exper. Therap., 1916, viii, 205.
(2) Cannon and Rapport: This Journal, 1921, lviii, 308.
(3) Cannon: Ibid., 1919, l, 399.
(4) Stewart and Rogoff: Ibid., 1918, xlvi, 92; 1920, lii, 521.
(5) Cannon and Rapport: Ibid., 1921, lviii, 338.
(6) Cannon and Hoskins: Ibid., 1911, xxix, 274.

THE "ALL-OR-NONE" PRINCIPLE APPLIED TO MAMMALIAN NERVES AND REFLEX-ARCS

J. M. D. OLMSTED AND W. P. WARNER

From the Department of Physiology, University of Toronto

Received for publication April 17, 1922

The place of the "all-or-none" principle in modern physiology has been admirably set forth by Martin (1). Many physiologists are now satisfied that the experiments of Lucas (2) and Adrian (3) demonstrate the validity of this principle, at least for the motor nerves of the frog. When, however, one compares conduction in a motor nerve trunk artificially stimulated by an electric shock with that over a reflex-arc, where the motor nerve is stimulated in a more natural fashion, the differences are most apparent. Sherrington (4) has devoted a large share of his book, *The Integrative Action of the Nervous System*, to these differences. Graham Brown (5) has written specificially on the application of the "all-or-none" principle to mammalian reflexes, and has concluded that "the discharge of the efferent neurone in a specific type of reflex activity has *not* the character of an 'all-or-none' response to graded stimuli." This is in accord with the statement made earlier by Sherrington (4) that there is "less close correspondence between the grading of the intensity of the stimulus and the grading of the intensity of the end effect" in reflex-arcs than in nerve-trunk conduction. Sherrington and Sowton (6) later showed that with single inductorium break-shocks of graded intensity applied to an afferent nerve-trunk, a more extensive gradation of flexor contraction is found than in the case of the same muscle stimulated directly through its motor nerve. Furthermore this grading of muscular contraction persists even when the afferent nerve is stimulated by break-shocks which are supramaximal for the same muscle directly stimulated through its motor nerve. It is therefore possible, according to these authors, for a reflex response to be much greater than the maximum contraction excitable in a muscle-nerve preparation. Surely, this would appear to be incompatible with the "all-or-none" principle.

A similar phenomenon has been reported by Forbes and Gregg (7). Instead of the usual myograph records these investigators employed the electrical responses of muscles and nerves as a criterion of activity. They concluded that all the fibers in a nerve-trunk are active when the action current has attained a maximum. Nevertheless they were able to observe both an increase in the intensity of the muscular response and also a spread in reflex activity involving more and more muscles, when the stimulus applied to the afferent nerve was increased beyond this maximum.

The reason for this apparent failure of the application of the "all-or-none" principle to mammalian reflexes might lie in the very character of mammalian nerves themselves. The nerves of the frog, a cold-blooded and more primitive vertebrate, are known to be much more resistant to stretching, cooling, removal of blood supply, etc., than are mammalian nerves. It is, however, generally assumed, but has not as yet been proven, that the "all-or-none" law applies to all vertebrate nerve fibers in general. If the assumption is made that mammalian nerves do behave in the same manner as amphibian nerves it might be that conduction through some part of the reflex arc does not obey the "all-or-none" principle. When one considers the essential parts of a reflex-arc, viz., the afferent nerve fiber, its synapses with internuncial neurones in the central nervous system, the internuncial neurones themselves, their synapses with the efferent neurones, and finally the effervent neurones composing the "final common path," one is inclined to assume conduction to be the same in all nerve fibers and to suspect only the synapses for irregularities in behavior. There is certain evidence that conduction in afferent fibers is similar to that in motor fibers. Forbes and Gregg (8) found that upon stimulating mammalian nerves, both sensory and motor, with break-shocks of increasing intensity, a maximal value of the action current was reached at about 47 units on the Martin scale. Stimuli of several times this value caused no further increase in the action current, though in response to the more powerful shocks the nerve no longer recorded simple action currents. The curves for stimuli above 47 units were always deformed as if a second or third propagated disturbance had passed along the nerve as a result of a prolonged local excitatory process at the point of stimulation. Adrian (9) also found that there was no essential difference between the response of a sensory fiber and that of a motor fiber since they have the same chronaxie.

On the other hand the synapse, i.e., the junction between one neurone and another, has been held responsible for nearly every irregularity in the physiology of the nervous system. The most profitable view of the synapse in our opinion is that of Lucas (2) who considers it to be of the same character as an area of decrement in a nerve due to narcosis, etc., in passing through which the strength of the propagated disturbance (nervous impulse) becomes diminished. Sherrington (4, p. 17) has summed up the possibilities of the synapse and considers that it may very well be responsible for the difference between reflex-arc conduction and nerve-trunk conduction. It is a fact that although the nervous system is histologically continuous throughout its entire extent, yet when a reflex impulse is intensified it does not spread to every part of the body, e.g., in the flexion-reflex the spread never extends to the muscles of the pinna of the ear. Sherrington's explanation is that the resistance to irradiation in different directions is referable to differences in the facility of conduction at different synapses (4, p. 154). This doctrine of "graded synaptic resistance" has been criticised by Forbes and Gregg (8, p. 227) who show that the idea as outlined by Sherrington is absolutely "incompatible with the 'all-or-none' principle."

After studying Adrian's experiments which are considered "proof" that conduction in the frog's motor nerve follows the "all-or-none" law, we became convinced that the same methods could be applied to a study of conduction in mammalian nerves and to a certain degree to conduction over reflex-arcs. There appear to be two main points to be proven: *a*, "The size of the propagated disturbance at any point in a nerve fiber depends only on the local condition of the fiber at that point and not on the previous history of the disturbance before it arrived there" (3, p. 460). The proof of this was accomplished in the frog's motor nerve by using as a measure of the intensity of the nervous impulse its ability to pass through a narcotized area. Adrian (10) narcotized simultaneously a section of one nerve, and two shorter sections of another nerve. The lengths of these sections were so chosen that the sum of the two small sections was just equal to the large one, but the two small sections were separated by a short length of normal nerve. If the size of the propagated disturbance is dependent on the strength of the original stimulus then both nerves should become sufficiently narcotized to prevent the nerve impulse from reaching the muscle at the same time. But if upon reaching an unnarcotized area where the nerve may regain its full powers of conducting an impulse, and if the energy of the propagated disturbance then returns to its

original value, the nerve with the two narcotized regions should continue to send impulses through to the muscle after the other had ceased. He found the latter to be the case. *b*, "In any nerve fibre under normal conditions there is an all-or-none relation between the strength of the stimulus and the size of the propagated disturbance which follows it" (3, p. 472). This was proven by finding the strength of stimulus necessary to give maximum and minimum contractions of a muscle-nerve preparation whose nerve was being gradually narcotized. Adrian found that the strength of stimulus necessary to affect the muscle did not rise gradually, but remained approximately constant until conduction failed, i.e., the strength of stimulus required to give a maximal contraction never rose, although the maximal contractions diminished in height as narcosis progressed. The strength of stimulus necessary for minimal contractions, however, did rise during narcosis and before the complete extinction of the propagated nervous disturbance it reached the same value as for a maximal contraction. This can only mean that the "effective strength of stimulus for any one nerve fibre remains absolutely unchanged until the moment when conduction in that fibre is completely suspended."

EXPERIMENTAL RESULTS. Our experiments were performed on decerebrate cats prepared according to Sherrington's method. The cut passed through the anterior corpora quadrigemina and no portion of the cerebral hemispheres remained. We found that if instead of checking at once the hemorrhage from the vertebral arteries we allowed a small amount of blood to escape and form a dense clot over the stump of the brain stem the preparation would remain in a good condition for a longer period than otherwise, in several cases for two days. It is of course necessary that the preparation be kept sufficiently warm. In nearly all the experiments the muscle used was the tibialis anticus, which can be readily freed from its insertion at the bony tubercle halfway between the point of the heel and the root of the first toe with minimum damage to circulation, etc., in order to allow the tendon to be attached to a lever. The muscle contractions were recorded isotonically. The motor nerve for this muscle is the peroneal, and the sensory nerve for reflex stimulation, the popliteal. The popliteal was severed near the knee and enclosed in Sherrington electrodes for stimulation in a central direction. We found that if the small blood vessels lying on this nerve could be preserved the nerve would sometimes remain sensitive over-night without changing the position of the electrodes, but that if we were unable to preserve this blood supply the

sensitivity of the nerve disappeared in an hour or so. In order to prevent interference from other muscles, all the hamstring and the femoral nerves were cut. In the case where the semitendinosus muscle was used, the motor nerve was the middle branch of the hamstring nerves and the sensory, the whole sciatic severed distal to the hamstring branches, in this case the whole femoral nerve was severed.

As the source of stimulation single condenser discharges were used. It is well known that in stimulation by electrical means both the difference of potential and the rate of discharge are factors in setting up a disturbance in a nerve sufficient to cause an impulse to pass along the nerve. By varying the resistance between the condenser and the stimulating electrodes the rate of discharge alone is changed. Varying the resistance is a very simple procedure, therefore it appears to be one of the best methods for varying the strength of stimulus since one factor only is changed, and that is done in a known quantitative manner. We have practically always employed this method in our experiments.

1. Motor nerves. Relation of strength of stimulus to its previous history. In order to narcotize portions of nerves, cups made in ebonite blocks were used. These cups were 5 mm. in depth and connected with the ends of the blocks by troughs 2.5 mm. deep and 3 mm. wide. One block 15 mm. square and 8 mm. deep contained a single cup of 9 mm. diameter. The other 25 mm. long, 10 mm. wide and 8 mm. deep held two cups each 4.5 mm. in diameter and separated from each other by a trough 10 mm. in length.

The motor nerve to be narcotized was laid in the troughs across the blocks without pulling or stretching. The troughs were then filled with vaseline so that only those parts of the nerve lying across the cups were bare. When the cups were in place a stimulus of sufficient strength was applied to the nerve to give just a maximum contraction of the muscle. There was then placed in the cups by means of a pipette 20 per cent alcohol (absolute alcohol in normal, 0.8 per cent saline). Enough alcohol was introduced to fill the cup completely and surround the nerve. A cover of mica was then placed over the cup in such a way that no bubble of air was included. This cover prevents evaporation, yet allows one to see into the cup, and is not so fragile as glass. Great care must be taken in the application of vaseline. There must be no vaseline on the length of nerve within the cup, yet everywhere else the nerve must be protected, especially between the two small cups, and there must be no leakage from the cups; care must also be taken that no blood clot forms in the cup for this protects the nerve and prevents

narcotization. At the instant of filling the cups a stop-watch was started and the nerve was stimulated usually every 30 seconds, sometimes every 15 seconds but not oftener. The record of the muscular contraction was taken on a slow-drum (fig. 1).

We found that if the blood supply to the nerves was in good order the nerve would begin to recover from complete narcosis within 5 minutes, the cups having been removed and the nerve bathed in warm Ringer's fluid. Furthermore the position of the electrodes could be left unchanged. Under these conditions we found that the height of the contractions was the same after narcosis as before. In the majority of experiments we therefore employed one nerve, using the two blocks

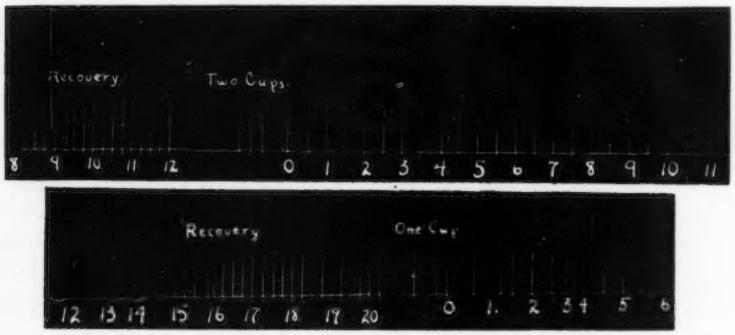


Fig. 1. Myograph records of contraction of tibialis anticus muscle in cat 26b. Electrodes for stimulation and cups for narcotization on the peroneal (motor) nerve. This is the fifth experiment on the same nerve without altering the position of the electrodes (see table 1). The block containing two cups was placed on the nerve after the recovery shown in the first part of the record. Alcohol was introduced at the moment indicated at *O*. The numbers indicate the time in minutes after the introduction of alcohol. The last response occurred at 11 minutes, there was no response at 11¼ minutes. The cups were removed at 12 minutes. The nerve was washed and the wound closed until 13½ minutes. At 14 minutes recovery began. Stimuli were sent in every 15 seconds for 6 minutes, the maximum having been reached. The block with the one cup was then put in place and alcohol added. The last response was at 5¾ minutes, none at 6 minutes.

alternately. We very often started the experiment with the block containing the one cup, for on the assumption that conduction in the nerve in the two cups would continue for a longer time than when the one cup was used, there might be some advantage for the first experiment tried. The nerve would be fresher, less liable to be damaged,

etc., than after the manipulation of putting the block in place and the recovery from narcosis. Nevertheless the results showed very consistently that conduction through the two cups continued longer than through the one. In table 1, which gives the results of our experiments on motor nerves, the numbers in parentheses indicate the order in which the trials were made. Thus in cat 26 two cups were first used, then the

TABLE 1

Effect of narcotizing the same length of a motor nerve in one section and in two sections. Electrodes on same motor nerve. The numbers in parentheses indicate the order in which trials were made. Change of electrodes or use of a new part of the nerve is indicated by a letter after the serial number of the experiment. The time in minutes from the beginning of narcotization to the last response is given

CAT	1 CUP, LAST RESPONSE AT	2 CUPS, LAST RESPONSE AT
	minutes	*minutes*
22	(2) 3¼	(1) 7
23	(1) 4½	(2) 3¼
24a	(1) 11¾	(2) 12½
24b	(1) 8¼	(2) 17
25	(1) 6¼	(2) 15½
	(3) 10	(4) 15½
	(5) 7½	(6) 9½
26a	(1) 9	(2) 9
		(3) 16
26b	(2) 6¼	(1) 10½
	(4) 4½	(3) 7¾
	(6) 5¾	(5) 11
26c	(2) 4¾	(1) 14¼
	(4) 5¾	(3) 8½
32	(1) 6	(2) 7
Average	6.6 minutes	10.9 minutes

one, then the two again, etc., and the stimulating electrodes were unchanged until after the sixth trial. In cat 23 there was probably a leakage of alcohol into the trough connecting the two cups though this was not noticed at the time. Taking the average of all the results conduction through the one cup lasted only 6.6 seconds, while through the two cups it remained for 10.9 seconds. Therefore for mammalian motor nerves as well as for the frog's motor nerves we can say that "the size of the disturbance at any point in a normal fibre depends only on the local condition of that point and not on the previous history of the disturbance."

2. Motor nerves. Maximal and minimal stimuli during narcotization. The experimental procedure for this part of the work was similar to that described in the previous section. Only the block containing the one cup was used. After the blocks had been set in place the strengths of stimulus required to give a maximal and a minimal contraction of the muscle were found. Twenty per cent alcohol in saline was introduced into the cup and the nerve was stimulated not oftener than every 15 seconds. The strengths of stimulus necessary to cause maximal and

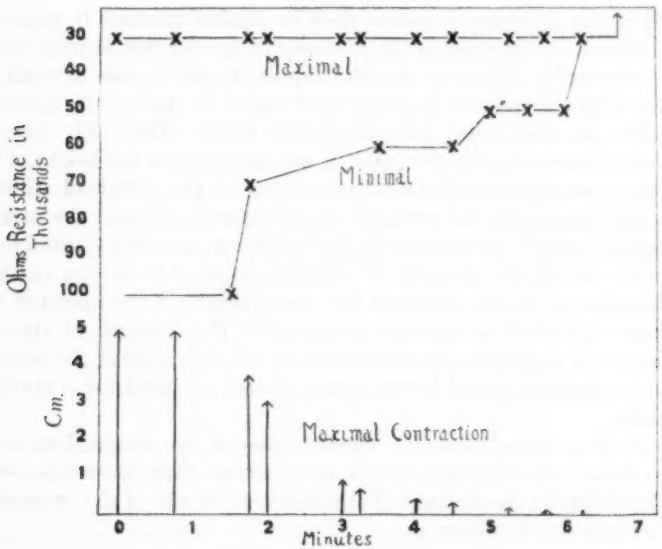


Fig. 2. Graph showing rise in minimal stimulus, constancy in maximal stimulus, and fall in maximal contraction during narcotization of motor nerve. Motor nerve stimulated. Abscissae mark time in minutes from beginning of narcotization; ordinates mark strengths of stimuli (resistance in ohms) and heights of muscular contractions (in centimeters). The miminal stimulus reaches the value of the maximal at 6¼ minutes. The nerve ceases to conduct after 6¾ minutes.

minimal responses were determined alternately until the muscle no longer responded even to what was previously a supramaximal stimulus.

A typical curve is shown in figure 2. From myograph records it appears that with the strength of alcohol we used narcosis begins to act within a very few minutes after its application. In control experi-

ments without alcohol the threshold for the minimal stimulus rose in a few cases until a constant was reached. In the majority of controls the threshold remained constant from the first. Therefore in a small proportion of our experiments with alcohol the first changes in the threshold stimulus may have risen due to "uncontrolled variations in the local excitability of the nerve." Lodholtz (11) has shown that such variations may be avoided in a frog's muscle nerve preparation if very great precautions are taken to prevent any change in the condition of the nerve in the region of the electrodes.

When the threshold stimulus rises in alcohol narcosis it might be due either to an increase in the intensity of the nervous impulse in the more susceptible fibers, so that the impulse is able to pass through the region of the decrement in them, or it might be due to the increased stimulus affecting other less susceptible fibers. That this latter is the case is shown by the fact that in our experiments the height of the maximal muscular contraction became less as the threshold stimulus rose, and increasing the strength of the stimulus beyond the original maximum caused no increase in the height of muscular contractions. In other words, the strength of stimulus required to give a maximal contraction never rose although the actual height of the maximal contraction decreased as narcosis progressed. The strength of stimulus necessary for minimal responses, however, did rise, until at the moment when conduction ceased it was equal to that required for a maximal stimulus.

From these experiments we conclude that in the mammalian motor nerve fibers, like the frog's motor nerve fibers, there is an all-or-none relation between the strength of stimulus and the size of the propagated disturbance which follows it.

3. Sensory nerves. One of the difficulties in investigating sensory nerves is in finding a satisfactory criterion of their activity. In using the muscular response in this capacity it is hard to obtain a maximal contraction without causing the whole body of the cat to move. Furthermore, changes in the condition of the central nervous system, such as those caused by a struggle on the part of the animal, pinching its toes, flicking its ears, etc., markedly influences the amount of muscular response obtainable by reflex stimulation. This is of course the phenomenon which Sherrington has called "spinal induction." We found, however, that if conditions were kept constant and decerebration had been so far posterior as to prevent entirely any mimetic movements on the part of the preparation we could obtain very consistent results (table 2,

especially cat 28). We found that the sensory nerves were more likely to lose irritability under the conditions of the experiment than motor nerves, yet we were compelled to use a 25 per cent solution of alcohol in saline in order to produce complete narcosis in the sensory nerves within a reasonable length of time. Thus it was sometimes impossible to use the same nerve for alternate trials with the one cup and two cup blocks as we did with motor nerves, since complete recovery of conductivity often did not return. In one case we dissected out the nerve

TABLE 2

Effect of narcotizing the same length of sensory nerve in one section and in two sections. Electrodes on same sensory nerve

CAT	1 CUP, LAST RESPONSE AT	2 CUPS, LAST RESPONSE AT
	minutes	*minutes*
22a	(1) 3½	(2) 6
22b	(1) 4½	(2) 5¼
24	(1) 6	(2) 15½
26a*	(1) 8½ (Right leg)	(1) 19½ (Left leg)
	(2) 4¾ (Left leg)	(2) 4¾ (Right leg)
27	(1) 9½	(2) 11½
28a	(1) 1	(2) 6½
28b	(1) 4½	(2) 5¼
	(3) 2¼	(4) 3
	(5) 6	(6) 8¾
	(7) 8½	(8) 15½
32†	2 (Right leg)	8 (Left leg)
34	(1) 2¾	(2) 5¾
Average.....	4.6 minutes	8.8 minutes

* Each side separately.

† Both sides simultaneously.

on the other side of the body and applied the other block to it for comparison with the first (cat 26). In only a few cases were we able to keep the position of the electrodes the same in different trials (cat 28). In an attempt to avoid these difficulties in general and also to have the same state of irritability in the central nervous system during both trials, we made a few preparations employing both legs simultaneously (cat 32).

Our results for sensory nerves are shown in table 2. We found that the average time for extinction of the impulse in a sensory nerve narcotized in one long section was 5 minutes, and that for a nerve narcotized

in two shorter sections, 8.7 minutes. These results are in agreement with those obtained for motor nerves.

For our second type of experiment we used cats which were practically without decerebrate rigidity, since we found in such preparations less likelihood of irregularities due to changes in irritability. A stronger stimulus was necessary to cause a maximal contraction than in the case of motor nerves, otherwise the procedure was the same. For the sake of comparison we used in one experiment single break-shocks from the secondary of an inductorium. In all respects the curves were of exactly the same nature as those for motor nerves.

4. Conduction over a reflex-arc. The experiments in section 3 might be classified as conduction over a reflex-arc, but in these experiments the cups were placed on the same sensory nerve that was stimulated, and we were careful to keep conditions so constant that we believe the response of the muscle was in all these cases a true index of the behavior of the sensory nerve. In order to test conduction over the reflex arc we stimulated the sensory nerve but placed the cups on the motor nerve. The nervous impulse therefore had to travel through at least one synapse before reaching the area of decrement in the narcotized motor nerve. Here again we found that under constant conditions exactly the same relationship held in each type of experiment which we had found for both motor and sensory nerves. Table 3 gives the results of the first type of experiment, and the curves in the second type of experiment are practically the same as those shown in figure 2. It therefore appears that under constant conditions the all-or-none principle holds for conduction over the reflex-arc in the mammal.

It is when conditions in the central nervous system are changing that we find behavior apparently at variance with the all-or-none principle. We have found that one of the best methods for changing the conditions of irritability in the decerebrate cat is to twist its head sharply to one side. This increases the decerebrate rigidity to a marked degree. In this state the preparation is very excitable and a slight stimulus will call forth a vigorous response. In properly decerebrated preparations excitability returns to its former condition within one minute after releasing the head. If we increased the excitability of the cat in this manner while conducting the second type of experiments reported in this section just after the moment when the original minimal stimulus had failed to give a response, we found that this original minimal stimulus now caused a vigorous response, and furthermore the following maximal stimulus caused a higher contraction than it had done just previously (fig. 3). A similar result could sometimes be obtained at the end of the

TABLE 3

Effect of narcotizing the same length of motor nerve in one section and in two sections, stimulus applied to sensory nerve

CAT	1 CUP, LAST RESPONSE AT	2 CUPS, LAST RESPONSE AT
	minutes	*minutes*
24	(2) $1\frac{1}{2}$	(1) $3\frac{1}{4}$
26*	(1) 3 (Right leg)	(1) 8 (Left leg) (2) $4\frac{1}{2}$ (Right leg)
29	(1) $5\frac{1}{2}$ (3) $3\frac{1}{2}$ (5) $3\frac{1}{2}$	(2) $4\frac{1}{2}$ (4) $6\frac{1}{2}$
30†	(1) $7\frac{1}{2}$ (Right leg) (2) $2\frac{3}{4}$ (Left leg) (3) $2\frac{1}{4}$ (Right leg) (4) $3\frac{1}{2}$ (Left leg)	(1) 11 (Left leg) (2) $3\frac{3}{4}$ (Right leg) (3) $2\frac{1}{4}$ (Left leg) (4) $4\frac{1}{2}$ (Right leg)
32†	(1) $4\frac{1}{2}$ (Left leg)	(2) $8\frac{3}{4}$ (Right leg)
Average.....	3.7 minutes	5.7 minutes

* Each side separately.

† Both sides simultaneously.

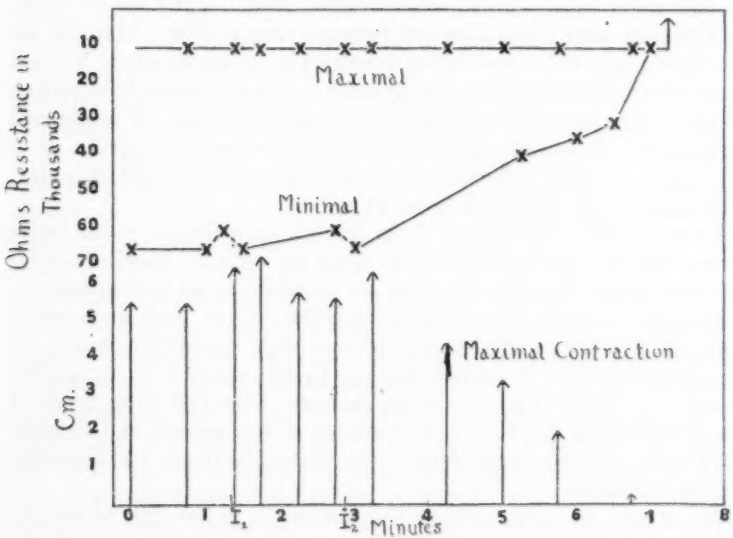


Fig. 3. Graph similar to figure 2. Sensory nerve stimulated, motor nerve narcotized. Minimal stimulus rose at $1\frac{1}{4}$ minute. Irritability artificially increased through spinal induction I_1 at $1\frac{1}{2}$ minute. Minimal stimulus returns to its former value, also height of maximal contraction increased. Similar effect at I_2. Otherwise results the same as in figure 2.

experiment at the moment when conduction in response to a maximal stimulus had just been extinguished for, by twisting the cat's head and thus increasing the irritability, this same strength of stimulus now caused a small contraction. This could be kept up for only a very few moments before all power of conducting an impulse disappeared. We tried this procedure in experiments where the sensory nerve was stimulated and the cups were on the sensory nerve, and found the same thing to be true.

Discussion. It would appear that there can be no question but that the motor and sensory nerves of the cat, and reflex-arcs as well, conduct nervous impulses according to the all-or-none principle, *provided conditions are kept constant, especially the state of excitability in the central nervous system.* The nearer one can reduce the cat to the conditions of the ordinary muscle-nerve preparation, the more exact is the application of the all-or-none principle. But with the exployment of excessive strengths of stimuli, the performance of any "voluntary movement" on the part of the preparation, the placing of a part of the preparation in a new position, etc., all result in a condition of increased excitability, so that subminimal stimuli become supraminimal, and a former maximal stimulus may cause a greater response than at first. Many of the discrepancies in reflex conduction described in the introduction, it seems to us, may be explained on these grounds. But it remains to be proven whether this increase in response during a condition of heightened irritability is due to changes in intensity of the nervous impulse, or to changes in extensity whereby more nerve fibers are brought into play, each acting at its maximum or not at all.

Lucas, Adrian, Forbes and Gregg in particular incline to the latter view, while Sherrington and Brown favor the former. Sherrington (4) has enumerated the ways in which the central nervous system may influence the response to afferent stimulation; *a*, by "successive spinal induction;" *b*, by "reinforcement" or immediate spinal induction; *c*, by the influence of the "tonus labyrinth." In our experiments stimulation of the contralateral hind foot is the example of the first; stimulation of the contralateral fore-foot is an example of the second; and twisting the head an example of the third. The mechanism of the first and third may be the same.

An attempt has been made by Sherrington (4, p. 120–124) to show in an experiment on the scratch-reflex that such spinal induction (using this term generally to include all three methods) is a matter not of extensity but of intensity. He considers that there are two possibilities,

either that particular groups of afferent fibers may be connected with separate groups of motor fibers in the final common path; or that the entire group of neurones composing the final common path may be connected with each group of afferent fibers. He regards the first as a case of extensity and the second, intensity. His proof that the second hypothesis is correct rests on the fact that although the flexor muscle fibers of the hip are able to respond to stimuli of a quicker rhythm than four per second, nevertheless stimulating two points on the skin alternately each with single shocks sent in at the rate of four per second produced no change in the rhythm of the scratch-reflex, but did produce a greater amplitude of the individual muscular contractions.

We are of the opinion that the scratch-reflex is too complex a phenomenon to be used to settle this point. In the first place, a stimulus consisting of a single shock never calls forth this reflex. There must always be summation of stimuli which in itself introduces complications. Second, the phenomenon is a rhythmic one involving alternate contraction and relaxation in a certain set of muscles, and this rhythm is unalterable, as Sherrington shows. Changing the intensity of the repeated stimuli, or changing the rhythm of the stimuli has no effect on the rhythm of the response. There must be, therefore, a rather complicated nervous mechanism involved in the rhythmic control of the scratch-reflex which is quite incomparable with that involved in a single flexion-reflex. If, now, one rules out the rhythmic character of the scratch-reflex which is without counterpart in the flexion-reflex, and considers what happens in the scratch-reflex, when the strength of stimulus is increased, or more than two points on the skin are scratched simultaneously, one finds that there is an increase in the amplitude of the individual beats. This is precisely comparable to what one finds in the flexion-reflex, and one can explain this effect on the ground that with increase in stimulus more fibers come into action. Sherrington has considered that this is a case of intensity, where all the neurones composing the final common path constitute together one almost unitary apparatus, so that stimulation of any one point in the whole receptive field can excite them all. As an example of the other type of reflex, the experiments of Camis (12) may be quoted. He found that maximal stimulation of two different nerves gave a greater reflex contraction than was possible by stimulating one of them alone. In fact, the peroneal and saphenous nerves are so independent in their action on the semi-membranosus muscle, that the myogram obtained by simultaneous excitation of these two nerves is equal to the sum of both myograms, no matter which of the two is stimulated first.

In either case whether it is possible for the whole final common path to be linked with one group of afferent nerves or only a part of it, we can see no objection to the assumption that the individual nerve fibers function on the all-or-none principle.

There appears, however, to be distinct difficulty with Sherrington and Sowton's (6) statement that a reflex response may be much greater than the maximum contraction excitable in a muscle nerve preparation. It is always assumed that when a maximal contraction is given by a muscle-nerve preparation all the nerve fibers have been stimulated. This is the very basis of all Adrian's experiments. If now by employing reflex stimulation there can be produced a greater contraction than when all the motor fibers are stimulated directly, the increase in response would at first sight appear to be possible only as an increase in the intensity of the nervous impulse. Forbes (12), however, has outlined a scheme in which he shows that this, too, may be a matter of extensity. There are many internuncial neurones between the afferent nerves and the neurones of the final common path. These may occur in chains of varying length so that all the impulses which leave the afferent neurone may not reach the neurones of the final common path at the same time. Thus some may arrive in the "supra-normal" phase found by Lucas (2) to occur in frog's motor nerve immediately after the end of the refractory phase. In such a case summation would occur and a greater contraction would result. The all-or-none principle does not claim that each nerve or muscle fiber always acts at the same maximum but that under a given condition each nerve and muscle fiber acts to its fullest capacity or not at all.

Although it is possible to explain theoretically this phenomenon observed by Sherrington and Sowton, in none of our experiments have we ever observed a greater response on the part of a single muscle to reflex stimulation than to direct stimulation of its motor nerve and it has always taken far less strength of stimulus to obtain the maximal response when the motor nerve was stimulated directly. Forbes and Gregg (7) have found that in using string galvanometer records of action currents as a criterion of activity reflex, responses in nerve to maximal stimulation were much smaller than the responses to direct stimulation.

Another apparently insurmountable obstacle in the way of the all-or-none principle lies in the interpretation of the phenomena of vision and other sensations such as touch, hearing, etc. Both Brown (5) and Forbes and Gregg (8) have been impressed with this difficulty. Since their papers were written there has appeared the record of a series of

most carefully worked out experiments on the blue arc phenomenon by Troland (13). He shows beyond question that visual responses do act on the "all-or-none" principle, and he makes several valuable suggestions as to the ways in which it is possible for other sensations to obey the same law.

SUMMARY

1. It is possible to apply to mammalian nerves the same type of experimentation as that by which Adrian has proven that conduction in the motor nerve of the frog obeys the all-or-none principle.

2. Conduction in mammalian nerves, both sensory and motor, and over the reflex-arc as well obeys the all-or-none principle if conditions are kept absolutely constant.

3. Any change whatsoever in the state of excitability or any alteration in the condition of the central nervous system alters the relationship between the strength of the stimulus and the amount of the end-effect in reflex-arc conduction.

4. The increased end-effect due to heightened excitability may be equally well accounted for on the supposition that more neurones are involved as on the supposition that the intensity of the nervous impulse may be increased. In other words, in spite of the peculiarities of conduction over a reflex-arc, it still may follow the all-or-none-principle.

BIBLIOGRAPHY

(1) MARTIN: This Journal, 1922, lix, 400.
(2) LUCAS: The conduction of the nervous impulse, 1917.
(3) ADRIAN: Jour. Physiol., 1914, xl, vii, 460.
(4) SHERRINGTON: The integrative action of the nervous system, 1906.
(5) BROWN: Proc. Roy. Soc., 1914, lxxxvii, B., 132.
(6) SHERRINGTON AND SOWTON: Jour. Physiol., 1914, xlix, 331.
(7) FORBES AND GREGG: This Journal, 1915, xxxvii, 118.
(8) FORBES AND GREGG: This Journal, 1916, xxxix, 172.
(9) ADRIAN: Journ. Physiol., 1919, liii, 70.
(10) ADRIAN: Journ. Physiol., 1912, xlv, 389.
(11) LODHOLTZ: Zeitschr. f. allg. Physiol., 1913, xv, 269.
(12) FORBES: This Journal, 1921, lvi, 300.
(13) TROLAND: Journ. Opt. Soc. Amer., 1920, lv, 160.

STUDIES ON ALKALIGENESIS IN TISSUES

II. Ammonia Production in Muscle during Contraction

OLIVE PEARL LEE and SHIRO TASHIRO

From the Biochemical Department, University of Cincinnati

Received for publication April 27, 1922

In the course of general wear and tear, all the tissues undergo a certain amount of protein decomposition, but how far functional activities of the body in general depend upon protein metabolism is a question not yet answered. In muscle, in spite of evidences brought out by Scaffadi (1), Burian (2), Tullio (3) and others (7) it is not yet conceded by all that its contraction involves a change in nitrogen metabolism. If there is any relationship between muscular activity and nitrogen metabolism one would expect to find some evidence of it in the urine. Failure to obtain such evidence, however, is not surprising, considering the many other factors of variability of urinary nitrogen.

The failure to obtain evidence of protein metabolism during muscular activity might be due to at least four difficulties. In the first place, the amount of metabolites might be exceedingly small. In the second place, the end products of metabolism of different proteins in the tissue might not necessarily be the same under all conditions. In the third place there might be mutual transformation among some of these possible metabolites. Finally, accurate methods of estimation of these different nitrogenous metabolites are not always available.

In the first study (4) on alkaligenesis of the tissues, we demonstrated that NH_3 is one of the functional products of the nerve. Even if we assume that NH_3 were a sole product of protein metabolism of muscle, the urinary or blood NH_3 alone as determined by ordinary methods could not be used as a criterion in deciding whether or not similar metabolism occurs in contracting muscle. Errors of determination of NH_3 in the body fluid, the transformation of ammonia into urea and vice versa, or slight decomposition of other nitrogenous constituents might easily mask a true functional variation. It is not surprising, therefore, to note that all the evidence so far recorded in the literature

of such nitrogenous metabolism was obtained not by examination of the urine but either in isolated muscle, or in blood which had been subjected to the least experimental disturbance and above all which had not been treated with such reagents as NaOH or $Ca(OH)_2$ to decompose ammonium salts.

We have undertaken an investigation to see whether or not NH_3 is produced during muscular activity. We wished to know:

1. Whether muscle behaves similarly to nerve in increasing the output of NH_3 during functional activity. A negative finding here would not rule out a possibility of protein metabolism during muscular contraction, but a positive result if proved to be due to a physiological process would be good evidence for it.

2. Whether a muscle contributed any basic substance to a salt solution in which it was immersed. Some investigators have studied the rate of CO_2 production of different tissues by immersing them directly in an indicator solution. In order to test validity of this method, the actual basic substances contributed by muscle had to be measured.

3. How large the amount of NH_3, if any, given off by muscle is. For in order to know the fate of NH_3 given off by nerves, and how far the body NH_3 influences acid base balance in the body, it is desirable to know the actual amount of the base contributed by all the tissues.

In answering these questions we endeavored to measure the exact amount of volatile base produced in muscles rather than the total ammonium salts present in the muscle before, during and after contraction. The present communication records the amount of ammonia gas given off by a muscle under various physiological conditions.

EXPERIMENTAL. *Method and materials.* Gastrocnemius muscles of the frog, *Rana pipiens*, kept in laboratory aquarium from one day to many weeks were used exclusively. The muscle was quickly removed after the animal was decerebrated, and placed in Ringer's solution, avoiding as much as possible bruising or stimulating by contact with rough instruments. The adhering liquid is thoroughly removed with filter paper, the muscle weighed on a counterbalanced watch glass, and replaced in Ringer's solution for two or three minutes in order to lessen any stimulating effect, as well as to enable us to start the respiration experiment at fairly uniform intervals after the time of decerebration of the animal and isolation of the muscle from the body. The muscle was placed on electrodes fitted in paraffined corks, and placed in pyrex test tubes, containing each 1 cc. of indicator acid solution (MB-

MR-acid solution). The muscles were suspended over the liquid, which they did not touch. The respiration was allowed to continue usually fifteen minutes except on special occasions where different periods of respiration were desirable. The volatile base, if any, absorbed by the MB-MR-acid solution was analyzed in exactly the same way as described in the previous paper on nerve, by titration.[1]

Although all the quantitative data recorded in the following pages were obtained by the titration method alone, we ran extensive preliminary experiments with Graves' reagent. It will be recalled that a dilute NH_3 solution, superimposed on Graves' reagent forms a white cloud ring at the junction of the two solutions.

RESULTS. *a. Resting and contracting muscle.* In order to eliminate as far as possible physiological variations of different animals, we have usually compared the two muscles of the same frog, one only being stimulated. Stimulation was applied directly to the muscle by means of a break induced shock just strong enough to give a vigorous contraction, the frequency being regulated to about 24 times per minute by a metronome. In this way ordinary fresh isolated muscles from a healthy frog could be kept contracting periodically for an hour or so. A detailed procedure of a typical experiment is exactly identical to that shown in table 3 in study I. In table 1 are given the results of quantitative estimations of NH_3 production in the muscle during rest and contraction. Only those cases where the muscles of the same frog were used simultaneously for resting and stimulation experiments are included in the table.

As usual, the individual variation is considerable, no doubt due to the error of method as well as physiological variation. In the muscles of the same frog one very rarely fails to see the increased NH_3 production during contraction. In the course of large numbers of experiments, occasionally we found that contracting muscle gave far less NH_3 than the resting. The most of these cases we believe were due to unexpected injury, or the physiological condition of the muscle was such that they could not eliminate lactic acid quickly enough (see page 249). The results show that the average gastrocnemius muscle of the frog, ranging from 17 to 21 cm. in length, gives off 3.8×10^{-7} grams of NH_3 without stimulation and 7.56×10^{-7} grams during contraction, calculated on the basis of 1 gram of the muscle and 15 minutes' respiration. Thus, during rhythmic muscular contraction, NH_3 production is nearly doubled.

[1] For detailed method see page 532, lx, this JOURNAL.

Tullio (3) could not demonstrate an increased NH_3 production in the blood of veins after vigorous contraction of muscles (man and animals), when he used milk of lime to displace NH_3 from the blood. By utilizing blood alkalinity[2] instead of $Ca(OH)_2$, he found 0.77 mgm. of NH_3 in 100 grams of the blood of man during rest, and 0.84 mgm.

TABLE 1

Amount of NH_3 given off by resting and contracting gastrocnemius muscle of frog, Rana pipiens

EXPERIMENT	TEMPERATURE	WEIGHT OF MUSCLE	TIME ELAPSED FROM DECEREBRATION OF THE FROG TO THE BEGINNING OF RESPIRATION	DURATION OF RESPIRATION	CONTRACTION	TOTAL NH_3 GIVEN OFF	AMOUNTS NH_3 GIVEN OFF, CALCULATED ON BASIS OF 1 GRAM OF THE TISSUE AND 15 MINUTES RESPIRATION
	degrees C.	*mgm.*	*minutes*	*minutes*		*grams*	*grams*
55	21.5	600	6½	15	+	7.5×10^{-7}	12.5×10^{-7}
		635	6½	15	−	2.5×10^{-7}	3.93×10^{-7}
59	23.5	905	7	15	+	4.8×10^{-7}	5.3×10^{-7}
		870	7	15	−	3.2×10^{-7}	3.67×10^{-7}
61	21.5	770	14	15	−	1.6×10^{-7}	2.07×10^{-7}
		795	14	15	+	4.0×10^{-7}	5.03×10^{-7}
62	21.5	560	5	15	+	5.0×10^{-7}	8.92×10^{-7}
		520	5	15	−	3.0×10^{-7}	5.76×10^{-7}
63	20.5	645	6	15	+	5.0×10^{-7}	7.75×10^{-7}
		650	6	15	−	3.0×10^{-7}	4.61×10^{-7}
64	24.0	1010	10	15	−	3.0×10^{-7}	2.97×10^{-7}
		1625	10	15	+	6.0×10^{-7}	5.91×10^{-7}

Average for contracting muscle.....7.56×10^{-7} grams.
Average for resting muscle.....3.83×10^{-7} grams.

during muscular activity. In dogs' blood 0.27 mgm. in resting and 0.37 mgm. during muscular exertion. This is by far a smaller increase than our finding. Experimental conditions are, however, not comp-

[2] From his orginal Italian article we were unable to get an accurate notion of just how this was accomplished. We take it, however, that he probably passed an inert gas or air freed from NH_3 and CO_2 through the blood until all the CO_2 was driven off and bicarbonate decomposed.

arable in the two cases. The important part of his conclusion is that the active muscle produces and eliminates more NH_3 in a given time than muscle in repose.

The most striking point of our result is that the muscle gives off far less NH_3 gas than does the nerve. As shown in table 2, the resting nerve produces thirteen times more NH_3 per unit weight than the resting muscle. During functional activity the nerve gives off fourteen times more NH_3 than the muscle. In other words, the amount of the base produced from the activated muscle is much smaller than the amount of acid produced under the same condition. $\frac{NH_3}{CO_2}$ in case of the nerve was $\frac{1}{17}$ by weight. In muscle, this ratio must be far less than $\frac{1}{100}$. Whetherthis means that the actual NH_3 production in

TABLE 2

Showing comparative amounts of NH_3 production in sciatic nerve and gastrocnemius muscle of frog, Rana pipiens

CONDITIONS	TISSUE	TEMPERATURE	AMOUNT OF NH_3 GIVEN OFF, CALCULATED ON BASIS OF 1 GRAM DURING 15 MINUTES	NERVE NH_3 / MUSCLE NH_3
		°C.	grams	
Resting	Nerve	20–24	51.2×10^{-7}	13.3
	Muscle	20–24	3.8×10^{-7}	
During functional activities	Nerve	20–24	108.8×10^{-7}	14.2
	Muscle	20–24	7.56×10^{-7}	

muscle is really smaller than that in the nerve, or that the greater part of the base is held in the muscle by non-volatile acid formed during its activity might be determined by stimulating muscle at the beginning of the respiration period and collecting the gas during a longer period of respiration so that the lactic acid might be given an opportunity to be oxidized more completely. The result of such experiments show (see table 3, page 250), that the actual production of NH_3 gas in the muscle is far less than that of the nerve.

b. Injured muscle. In these experiments, muscles were first "mashed," but not ground, by rough instruments, and then placed in the respiratory chamber. The results are exactly the same as were obtained with the nerve. In all cases, excepting two, no NH_3 is produced from the severely injured muscle during first 15 minutes respiration. In two exceptions, muscle 74 gave 6.2×10^{-8} grams as muscle 76 gave 4.7

$\times 10^{-8}$, both of which are less than one-tenth of that of the normal muscle.

c. Tetanized muscle. The muscle was stimulated with weak tetanizing current from induction coil, having the frequency of about 60 times per second. During the whole respiratory period the muscle is thus kept in complete tetanus. In every case muscle thus treated gives off no NH_3, as tested with our method. The results fail to show whether this muscle is producing less, or more NH_3 than the resting nerve. Considering the well-known increase in acidity of the muscle during tetanus, this failure can be ascribed to the non-volatile acid holding the NH_3 if any is produced in the muscle (see table 3). The important point is, however, that for experiment with contracting muscle the conditions tending to produce fatigue or tetanus must be avoided as much as possible. These results show beyond doubt that our method of titration actually measures a volatile base forming compound.

d. NH_3 production in muscles on standing under various conditions: 1. NH_3 production at successive intervals of survival. In these experiments a series of 4 test tubes containing exactly the same amount of MB-MR-acid solution was prepared. The two muscles were prepared from the same frog, one placed in the first tube and the other in the next, the remaining two tubes of the group acting as controls. The muscle in the first tube was stimulated at definite intervals for 15 minutes. At the end of 15 minutes, after the usual procedure, both muscles were transferred into corresponding tubes of the second group, no more stimulation being applied to the stimulated muscle during subsequent respiration.

The results of titration of each of these tubes show that during the first 15 minutes the NH_3 production is usually at the maximum in both resting and contracting muscle. During the next 15 minutes following contraction, the stimulated muscle gives a still greater amount than the unstimulated muscle. In the majority of cases, even at the end of the third 15 minutes, the muscle once activated produces a little more NH_3 than the unstimulated. The result of these experiments are given in figure 1.

The slight rise during the fourth period is not significant. The fact is, the majority of muscles give less NH_3 than that of the preceding period. The average value given here has been greatly influenced by the muscle of frog 57, which gave 11.9×10^{-7} grams for the resting and 13.7×10^{-7} grams for the one contracted. If we omit these two the

average will be much lower as indicated by the dotted lines. Just when the curve begins to rise is not the question we investigated carefully. For such an experiment one should control experimental condi-

TABLE 3

NH_3 production from the muscle determined during longer period of respiration

NUMBER OF EXPERIMENT	TEMPERATURE	FROG		WEIGHT OF MUSCLE	TIME ELAPSED FROM DECEREBRATION OF FROG TO BEGINNING OF RESPIRATION	RESPIRATION	TREATMENT	TOTAL NH_3 GIVEN OFF	NH_3 PRODUCED, CALCULATED ON THE BASIS OF 1 GRAM MUSCLE AND 15 MINUTES RESPIRATION
		Sex	Size						
	degrees C.		*cm.*	*mgm.*	*minutes*	*minutes*		*grams*	*grams*
81	28.5	♀	21	675	5½	129½	S.	16.1×10^{-7}	1.83×10^{-7}
				690	5½	129½	T.	4.9×10^{-7}	0.548×10^{-7}
83	28.5	♂	20	580	10	127	S.	7.7×10^{-7}	1.03×10^{-7}
				620	10	127	T.	5.6×10^{-7}	0.713×10^{-7}
85	29.5	♂	20	785	13	127	E.	1.4×10^{-7}	0.14×10^{-7}
				780	13	127	R.	9.1×10^{-7}	0.913×10^{-7}
86	29.5	♀	20	796	7	127	R.	4.9×10^{-7}	0.48×10^{-7}
				800	7	127	E.		
90	28.0	♂	20	724	11	120	S.	4.9×10^{-7}	0.558×10^{-7}
				756	11	120	T.	2.1×10^{-7}	0.225×10^{-7}
91	28.0	♀	20	655	5	108	R.	8.4×10^{-7}	1.19×10^{-7}
				665	5	108	E.	1.4×10^{-7}	0.194×10^{-7}

S. = Muscle is stimulated by break induced shock during first 15 minutes respiration.

T. = Muscle is tetanized by weak induced shocks during first 15 minutes respiration.

E. = Muscle is crushed before placing in the respiratory chamber.

R. = Unstimulated muscle.

Average S. = 1.13×10^{-7} grams.

T. = 0.49×10^{-7} grams.

E. = 0.167×10^{-7} grams.

R. = 0.862×10^{-7} grams.

tions more rigorously, as the temperature effect on the death point of the muscle and the NH_3 production is quite divergent. The point of importance is that NH_3 when measured as a gas, does not show a

steady increase during a short interval of survival as one would expect, if it is due to the post-mortem decomposition of urea, or other nitrogenous compounds.

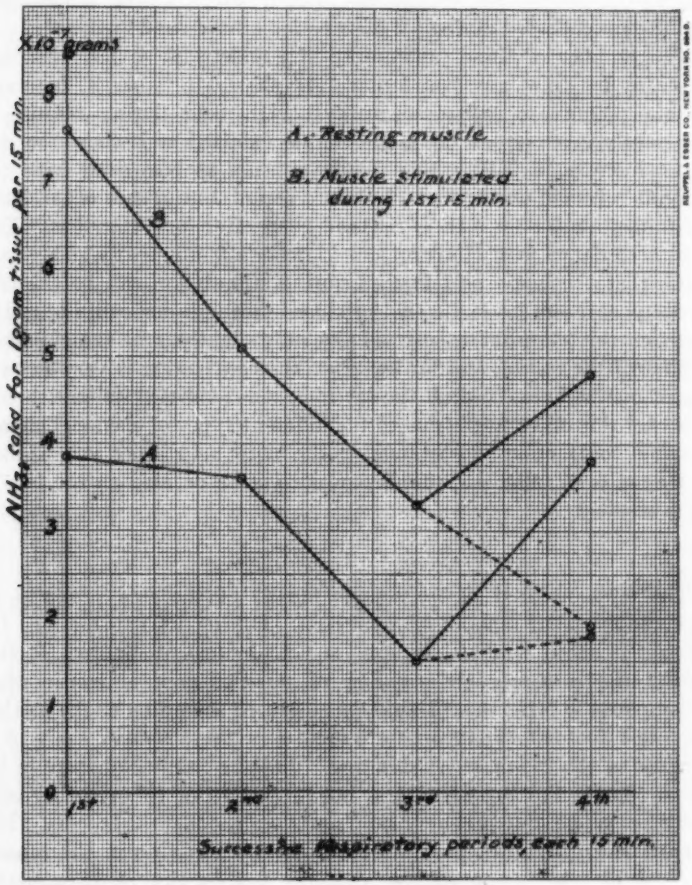


Fig. 1

2. Experiments with longer intervals of respiration. In order to see what is happening to NH_3 production during tetanus and injury where, even if the gas may be formed, the acid will be able to hold it, the muscles variously treated were allowed to respire for a longer period.

In the case of contracting and tetanized muscles, the muscles were stimulated in the respiratory chamber during the first 15 minutes and let stand over 2 hours and the total NH_3 given off was estimated. In the case of trauma, the muscle was injured before placing in the tube, and the gas collected during a 2-hour period or more and analyzed.

The number of these preliminary experiments is so few that one hesitates to draw any conclusion. As shown in table 3, however, certain points are rather suggestive. NH_3 held during tetanus is apparently given up as the lactic acid is oxidized as is shown by the slight increase in NH_3 which finally appears as compared with the amount collected during first 15-minute period, but in case of mechanical injury almost no NH_3 is released even after long standing. This is in accordance with Hopkins and Fletcher's finding (6) which establishes that the lactic acid formed during stimulation can be oxidized or disappear, but the lactic acid produced during injury will not. The fact that the tetanized muscle will give only slightly more than one-half of that of the resting and far less than the contracting is very surprising, if one assumes that all the lactic acid is oxidized within 2 hours. Whether this difference is due to the different types of chemical change between contraction and tetanus, or the lactic acid is not yet oxidized in case of once tetanized muscle can not yet be decided on the face of these results.

CONCLUSION

The fresh isolated gastrocnemius muscle of the frog produces an exceedingly small amount of ammonia. During its contraction, the amount is about double. In spite of Gad-Andersen's finding (5) that during the post-mortem standing of muscle, urea is continuously decomposed to NH_3, the NH_3 gas we measured according to the method described does not owe its origin to urea, since there is no evidence of an increase of the gas on short standing. Judging from the nature of the curve (fig. 1) this muscle NH_3 is not probably due to any other post-mortem change, but probably represents a physiological process. It is a partial expression of protein decomposition going on in the muscle, and can be taken as an additive evidence to show that nitrogen metabolism is involved during its functional activity.

According to Burian, hypoxanthine at first increases on stimulation of muscle. If this base is produced from adenine, we expect a simultaneous production of NH_3. NH_3 produced during functional activity in the muscle, however, is not likely due to the purine metabolism, considering the analogous case of the nerve fiber, where one would

expect the least possibility of such a metabolism. An analogy, however, does not prove any thing.

According to our results, the total muscles of a human being weighing[3] 150 pounds should give off 1.043 grams of NH_3 per day if we calculate it on the basis of frog's gastrocnemius muscle and, at the maximum, the activity of all muscles in the body can never give beyond 2 grams of the gas. This calculation is given merely to show that muscle NH_3 must be transformed to something else, probably urea. Under this condition, one would easily fail to detect an increase of N output in urine during severe muscular activity, no matter to which compound NH_3 might be transformed.

As shown in table 2, muscle gives off the base-forming substance far less than does nerve. Since we have evidence to show that the various other tissues too give off an entirely different amount of this basic compound, it is an exceedingly unsafe procedure to depend on a method of estimation of CO_2 by immersing the isolated tissues directly in an indicator solution and by measuring an amount of an increase of H^+ concentration, or by determining rate at which the solution reaches a definite pH.

SUMMARY

1. A resting gastrocnemius muscle of the frog, *Rana pipiens*, gives off 3.83×10^{-7} grams NH_3, calculated on the basis of 1 gram, of the tissue and 15 minutes of respiration. During 360 contractions it gives off 7.56×10^{-7} grams NH_3. One gram of muscle, therefore, produces approximately 1×10^{-9} grams more NH_3 during a single contraction.

2. Tetanized and injured muscles do not give off any NH_3, probably due to simultaneous production of a non-volatile acid. NH_3 held by tetanized muscle will be released if we let it recover. Injured muscle will not.

3. For exactly the same unit, muscle gives $\frac{1}{14}$ as much NH_3 as nerve. This shows a danger of erroneous conclusion if for estimation of CO_2 of the tissues one depends upon the direct indicator method alone, without eliminating the presence of the base-forming substance in the solution.

BIBLIOGRAPHY

(1) Scaffadi: Biochem Zeitschr. 1911, xxx, 473; xxxiii, 247.
(2) Burian: Zeitschr. f. Physiol. Chem., 1905, xliii, 532.
(3) Tullio: Arch. fisiol 1912, x, 71.
(4) Tashiro: This Journal, 1922, lx, 519.
(5) Gad-Andersen: Journ. Biol. Chem., 1919, xxxix, 267.
(6) Fletcher and Hopkins: Journ. Physiol., xxxv, 1906, 261.
(7) Cathcart: The physiology of protein metabolism, ix.

[3] Forty-two per cent of the human body is considered to be made of muscles.

THE PRODUCTION OF ADRENAL DISCHARGE BY PIQÛRE

R. CARRASCO-FORMIGUERA

From the Laboratories of Physiology in the Harvard Medical School

Received for publication April 28, 1922

Eckhard (1) and others proved Bernard's (2) contention that puncture of the floor of the fourth ventricle sends impulses along the splanchnic nerves. Later evidence first suggested and then proved beyond doubt that direct (3) or reflex (4), (5), (6) splanchnic stimulation produces the discharge from the adrenals of a product that reaches the arterial blood in sufficient amount to exert on other organs an action identical to that of adrenalin.[1] From these two facts it was natural to infer that Bernard's piqûre produces a discharge of epinephrin. Whether such is the case or not has been the subject of much work and still more discussion. The purpose of this paper is to contribute to the settlement of this question.

Mayer (9), Kahn and Starkenstein (10) and Biberfeld (11) have reported failure to obtain piqûre glycosuria in adrenalectomized animals, and have inferred that in normal animals the puncture elicits a discharge of epinephrin which is responsible for the observed glycosuria. In these experiments, however, the possibility that the splanchnic nerves had been damaged during adrenalectomy was not excluded, nor the possibility of impairment of renal function by the general condition of the animal, nor was any account taken of the glycogen content of the liver, a point which, as recalled by Stewart and Rogoff (12), is of essential importance. The results of those experiments are, therefore, not conclusive.

Jarisch (13) found that piqûre produces hyperglycemia in rabbits with hepatic nerves cut, provided one adrenal and its nerves are intact, whereas it fails to do so if, with hepatic nerves intact, the nerves to both

[1] Everybody, except Popielski (7), admits an epinephrin discharge elicited by direct splanchnic stimulation. Gley and Quinquaud (8) claim that the discharged epinephrin does not reach the arterial blood in sufficient amount to influence other organs. Both Popielski's and Gley and Quinquaud's positions are untenable in view of at least a part of the existing evidence.

adrenals have been cut. These results would almost settle the problem if they were unquestionable, but they are not. A careful reading of this work shows many weak points among which are the following: the animals were under ether anesthesia, which in itself produces hyperglycemia of variable degree; in one place a blood sugar figure of 0.164 per cent was called a hyperglycemia and in another place 0.167 per cent was said to be "the upper limits of the normal contents;" and finally, only one determination of blood sugar was made after the puncture, with no previous determination. On the other hand, Stewart and Rogoff (12) have proved that piqûre may produce hyperglycemia in experimental conditions under which the possibility of an epinephrin discharge seems to be excluded. Similar results have been obtained, although with questionable methods, by Wertheimer and Battez (14) and by Freund and Marchand (15). The existing evidence on the behavior of blood sugar and glycosuria after puncture throws no light, therefore, on the question under consideration.

Kahn (16), (17), (18) Starkenstein (19), Negrin (20), Negrin and Brücke (21) and Borberg (22) found after piqûre a marked decrease of the chromaffin reaction of the adrenal medulla. This shows that something happens there as a result of piqûre, but does not prove that what happens is an epinephrin discharge.

Kahn (16), (17), (18) also found that the actual epinephrin content of the adrenals is diminished by piqûre; his quantitative method was criticised by Popielski (7), but it is not necessary to discuss this criticism, since Kahn's result was confirmed by Sopeña (23) using a quite different method—that of Folin, Cannon and Denis (24). Kahn's and Sopeña's observations very strongly suggest liberation of epinephrin into the blood, the only possible alternative being the very unlikely one suggested by Popielski that epinephrin is destroyed *in situ*. Kahn also found that piqûre induced histological changes in the adrenal medulla, similar to those observed in other glands after secretory activity, e.g., protoplasmic vacuolization and disappearance of protoplasmic granules. This suggests epinephrin secretion. Nothing, however, in all these experiments of Kahn proves that the epinephrin which may have disappeared from the adrenals has reached arterial blood in sufficient amount to affect other organs.

Neubauer (25) noted that piqûre was followed by oscillations and a prompt increase of blood pressure followed by a gradual decrease; about three hours later a new increase occurred which he attributed to epinephrin. He presented no evidence for this assump-

tion. Trendelenburg and Fleischauer (26) observed that in deeply urethanized rabbits piqûre caused no increase of blood pressure and therefore inferred that there could be no discharge of epinephrin; this inference is not justified, since it is possible that adrenal secretion may be accompanied by conditions which prevent the appearance of its usual pressor effects. Indeed, after piqûre I have seen a fall of blood pressure attending unmistakable evidence of increased epinephrin in the blood, as will be described later. Furthermore, Trendelenburg and Fleischauer's observations are contradicted by a long series of very careful experiments conducted by Negrin and Hernandez Guerra (27). These observers show that, as a rule, especially if the vagi have been cut, as suggested by Pi Suñer,[2] piqûre results in a definite and lasting increase of blood pressure. Similar results have been obtained by Cervera (28). Since in these experiments the influence of vasomotor stimulation was not excluded, they cannot be considered proof of epinephrin discharge.

Watermann and Smit (29) found that the undiluted serum from 4 cc. of blood taken from two rabbits the day after puncture, caused in 20 to 30 minutes a noteworthy midriasis of the isolated frog's eye, used according to the Meltzer-Ehrmann technic (30). The same serum diluted 1:2 was ineffective. Any conclusion drawn from such meager results obtained by a highly questionable method would be unreliable. Kahn collected mixed blood from the inferior vena cava and the adrenal veins by a device similar to that of Cannon and De la Paz (31) and tested its serum by means of the Laewen-Trendelenburg preparation (32); he found that the serum of the blood collected after piqûre caused a much more marked vasoconstriction than did that of the blood taken before. Kahn interpreted this result as evidence of an increased output of epinephrin elicited by puncture. In criticism of this interpretation Stewart and Rogoff (12) pointed out that a decrease in blood flow would determine a higher concentration of epinephrin in the cava blood without an actual increase above what they claim to be the normal constant output. The possibility that blood pressure may fall and that blood flow may become retarded as a result of piqûre would render this criticism especially pertinent. Negrin (33), using as a test object the so-called denervated eye, according to the technic of Joseph and Meltzer (34), obtained results indicating that after puncture epinephrin discharged from the adrenals reaches the eye in sufficient amount to produce the so-called "paradoxical reaction." An important objection to these experiments is that the so-called denervated eye is not at all a denervated

[2] Personal suggestion to Negrin.

organ (4); piqûre perhaps more than other procedures might produce an inhibition of the tonic action of the third nerve, thus accounting for the observed reaction. Elliott (35), as a result of experiments in which after adrenalectomy he still obtained an incomplete paradoxical reaction on splanchnic stimulation, suggested that certain metabolites may influence the so-called denervated eye, although to a slighter degree than does epinephrin.

Finally Houssay and Cervera (35), using the method of Anrep (37) and working on dogs with the hind limb denervated and in most cases with the spinal cord severed below the twelfth dorsal vertebra and with the vagi cut, found that piqûre during a block of the normal venous outflow from the adrenals resulted only in a passive dilatation of the denervated limb due to increased blood pressure; and that when the venous flow from the adrenals was released some 2 or 3 minutes after the puncture a contraction of the denervated limb was obtained. Anrep's method or, in general, any other method involving the use of a denervated organ *in situ*, has been criticised by Stewart and Rogoff (12), who claim that the changes observed in the denervated structure may be brought about without any increase in the epinephrin output, either by a faster flow of blood through the part (due to increased blood pressure and vascular relaxation), carrying more epinephrin per minute through the vessels; or by a higher concentration of epinephrin in the blood reaching the denervated area because less blood from the constricted splanchnic vessels mixes with the blood from the adrenals. It has been shown that these theoretical possibilities play no part in the results obtained with one denervated organ—the denervated heart (4), (5) but since control experiments have not been done for the denervated limb, results obtained by this method remain under the shadow of Stewart and Rogoff's criticism. This comment applies to all of Houssay and Cervera's results because the increase of blood pressure and the constriction of the splanchnic area may last after puncture more than the 2 or 3 minutes during which these investigators keep clamped the veins from the adrenals.

We see that a number of facts have been collected that more or less strongly suggest that epinephrin is discharged as a result of piqûre. Some of these facts especially those found by Negrin (33) working with the denervated eye, and by Houssay and Cervera (36) employing the denervated limb, and, to less extent, some of those reported by Kahn (16), (18), and by Sopeña (23) taken singly or in combination, leave little doubt that puncture elicits an epinephrin discharge. It must be

admitted, however, that until control experiments prove the unsoundness of the objections to the methods employed, this conclusion cannot be regarded as unquestionable and final. For that reason I thought it worth while to apply still another method to the study of the question.

After Hunt (38), Levy (39), Gasser and Meek (40) and Anrep (37) had observed incidentally that the heart, disconnected from the central nervous system, beats faster under experimental conditions in which circulating epinephrin would probably be increased, Cannon (41) suggested the employment of the completely denervated heart to demonstrate an increase of epinephrin in the circulating blood. That this preparation is thoroughly reliable for the purpose in question has been clearly proved by Cannon and Rapport (5). This method was used in the present work.

All my experiments were done on cats, anesthetized either with a mixture of chloralose (0.03 gram per kilo) and chloral (0.5 gram per kilo) and morphine (0.0005 gram per kilo) given intraperitoneally, or first with ether and then with chloralose given intravenously (same amount) or first with ether and then with chloralose intravenously and chloral and morphine intraperitoneally, or with ether alone. The heart was denervated according to Cannon's directions (4); artificial respiration, started previous to opening the thorax, was continued until the end of the experiment in order to prevent any respiratory disturbance that might arise from injury to the medulla and mask the changes of heart rate properly due to piqûre. Blood pressure and heart beats were recorded from a femoral or carotid artery; time was recorded in 5-second intervals. Piqûre was performed by an adaptation to cats of Eckhard's technic for rabbits (1).

Perforation of the dura, with the necessary preliminary operation, always caused a more or less marked drop of blood pressure. In all except one animal (which had been used for other purposes) the blood pressure previous to that operation was above 100 mm. Hg. In one case, after the preliminary operation and before the puncture, the pressure went as low as 44 mm. Hg. In some instances, although having decreased, the pressure remained above 100 mm. Hg. Occasionally manipulation of the instrument near the medulla, just before the puncture, produced a new drop in the blood pressure. For this reason the blood pressure readings about 10 seconds before puncture are given for comparison with those following. In some cases, in which there was a great cardiac acceleration, irregularity of the heart beat (apparently heart block) was observed during a period of 5 to 10 seconds. The

heart rate per minute is determined by counting the number of beats in 5 seconds.

1. Piqûre increases the rate of the denervated heart. In a series of four animals puncture was performed after only the preparatory procedures. The main results of these experiments are summarized in table 1, a portion of the record obtained in experiment 2 is reproduced in figure 1. In three of the four experiments the first attempt at puncture was followed by a rise of blood pressure and a very remarkable increase of the heart rate. In the fourth animal the first puncture was

TABLE 1

Changes in heart rate and blood pressure produced by piqûre

NUMBER OF EXPERIMENT	CONDITIONS ABOUT 10 SECONDS BEFORE PUNCTURE		MAXIMAL CHANGES WITHIN 1 MINUTE AFTER PUNCTURE		CHANGES 1 MINUTE AFTER PUNCTURE		REMARKS
	Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure	
1	150	34	+48	+50	+45	+36	Piqûre
2	176	99	+64	+51	+52	+35	Ibid.
3	159	54	+63	+129	+51	+80	Ibid., 1st puncture
	184	70	+22	+65			Ibid., 2nd puncture
	180	75	+30	+65			Ibid., 3rd puncture
	180	55	+18	+12			Puncture of medulla below 4th ventricle
	180	48	+36	+42			Transection of medulla below 4th ventricle
4	208	82	0				Piqûre, 1st puncture
	208	78	+ 4				Ibid., 2nd puncture
	212	90	+52	+65	+16	+13	Ibid., 3rd puncture

quite ineffective so far as blood pressure and heart rate were concerned; a second attempt was followed by an insignificant increase of the heart rate and a very slight rise of blood pressure; a third puncture gave a result of the same order as that seen in the other three animals. The final success seems to indicate that the earlier attempts did not strike the right point. On the other hand, experiment 3 shows that, at least in some cases, it is possible to obtain more or less pronounced rises of blood pressure and increases of heart beat from successive punctures and other injuries of different points between the front edge of the floor of the fourth ventricle and a region in the medulla well below the ven-

Fig. 1. Record of beat of the denervated heart in a piqûre experiment (no. 2, table 1). The arrow marks the time of puncture. The original heart rate was 176; 40 seconds after puncture it was 240; and then it slowly decreased. The blood pressure rose sharply immediately after puncture, was stationary, and then rose again, as the heart was accelerated. Time line shows here and in other records, 5 seconds and zero blood pressure.

tricle. From the results of these experiments the conclusion may be drawn that puncture of different points in the floor of the fourth ventricle considerably increases the rate of the denervated heart.

2. Piqûre increases the rate of the denervated heart after section of the hepatic nerves. After Cannon had shown that in some cases the effect on the denervated heart produced by direct or reflex stimulation of the splanchnics was due only to a discharge of epinephrin from the adrenals, Gley and Quinquaud (42) reported one case and Stewart and Rogoff (43) a number of cases in which such stimulation resulted in increased heart rate even though the possibility of epinephrin discharge into the general circulation had been more or less completely excluded. Cannon and Rapport (5) confirmed this observation and proved that the effect did not take place if the nerves around the hepatic artery had been severed. Cannon and Uridil (44) then showed that acceleration of the heart could be evoked by a discharge from the liver induced by stimulating the hepatic nerves. A necessary step, therefore, in ascertaining whether there is an adrenal factor in the increased rate of the denervated heart, elicited by piqûre is to exclude the possible hepatic factor. Accordingly, in a second series of experiments, besides the preliminary operations described above, all the nerve strands accompanying the duodeno-hepatic artery were cut.

The main results of the series of experiments are summarized in table 2. These results offer some noteworthy features. In the first place, the cardiac acceleration, although still considerable, is, as a rule, smaller than that observed in the first series.

Still more marked differences are observed in the effects on blood pressure; in some cases the rise is very small, in some it fails altogether, and in others there is an actual drop. The same was true for experiment 4 of the first series, but in all the successful cases of that series puncture was followed immediately by a sharp jump of blood pressure followed by a more or less pronounced stationary period and then by a new rise, as may be seen in figure 1. In the present series, however, even when there was an important rise, as in experiments 9 and 13, it was more gradual. Whether all this is due to the elimination of a possible hepatic factor, or to impairment of splanchnic activity brought about by opening the abdomen or handling its contents, or whether it is a mere chance, dependent on the central points affected by puncture, has not been determined. To settle the question would require many more experiments than we have performed.

A third and very interesting peculiarity is that in some cases, with no rise of blood pressure, or even a decrease, there is a remarkable increase of the heart rate, showing that under certain circumstances one of the phenomena normally elicited by splanchnic stimulation may appear while another does not. Indeed, an antagonistic phenomenon may take place. Figure 2 illustrates this type of reaction.

It is clear that piqûre produces a remarkable increase of the rate of the denervated heart, even after an operation that eliminates acceleration by the possible hepatic factor evoked by direct or reflex splanchnic stimulation.

3. Piqûre does not result in increased heart rate when, after exclusion of the hepatic factor, one adrenal is removed and the other is completely tied off. As previously stated, the experiments of Cannon and Rapport (5) and Cannon and Uridil (44) proved that acceleration of the denervated heart by direct or reflex splanchnic stimulation was due only to adrenal discharge and to the action of an hepatic factor which can be excluded by cutting the hepatic nerves. If these facts are true also for splanchnic stimulation induced by piqûre, the foregoing experiments conclusively prove that piqûre produces a discharge of something from the adrenals that reaches the arterial blood and increases the rate of the denervated heart. But the objection might be raised that piqûre may bring into play some unknown factor that is not influenced by direct or reflex splanchnic stimulation. To check this possibility an experiment was done in which, after severing the hepatic nerves, the left adrenal was extirpated and the right was completely tied off without being removed. Under these circumstances, piqûre was followed by a maximal increase of two heart beats per minute, accompanied by a rise of blood pressure of 10 mm. Hg. To rule out the possibility that this negative result might be due to failure to hit the right point (see exper. 4, punctures 1 and 2, in table 1), the floor of the fourth ventricle was stimulated with a moderate tetanizing current, such that even if the electrodes were not applied exactly to the right area, the current could hardly fail to affect it by spreading. The result was a rise of blood pressure of 24 mm. Hg. accompained by reduction of the heart rate by 4 beats per minute. This kind of stimulation had been found to be effective even when performed after one or more punctures in some of the animals of the preceding series, including experiment 2 in which splanchnic activity seemed to be considerably impaired. In these animals the possibility of a discharge from the adrenals was not prevented. The main results of these experiments and those of the present experiment are given in table 3.

Fig. 2. Record of beat of the denervated heart in a piqûre experiment performed after cutting the hepatic nerves (no. 5, first puncture, table 2). The arrow marks the moment of puncture. Before puncture the heart rate was 132; 30 seconds after puncture it was 159. One minute after puncture it was still 150. The cardiac acceleration was accompanied by a decrease of blood pressure.

Thus when both the adrenal and hepatic factors were excluded, it was found impossible to increase the rate of the denervated heart either by puncture or by faradic stimulation of the floor of the fourth ventricle.

4. If after exclusion of the hepatic factor, the venous exit from the adrenals is temporarily occluded, piqûre is not followed by an increased heart rate, but the rate increases when, some time after the puncture, the venous block is removed without any other change in circulatory conditions. The experiment described in the previous section was not repeated because the objection might be raised that the negative results were perhaps due to impairment of some unknown extra factor by operation

TABLE 2

Changes in heart rate and blood pressure produced by piqûre after cutting the hepatic nerves

NUMBER OF EXPERIMENT	NUMBER OF PUNCTURE	CONDITION ABOUT 10 SECONDS BEFORE PUNCTURE		MAXIMAL CHANGES WITHIN 1 MINUTE AFTER PUNCTURE		CHANGES 1 MINUTE AFTER PUNCTURE	
		Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure
5	1	132	100	*+27*	−28	+18	−16
	2	138	95	*+35*	+20	+24	+ 8
	3	135	70	*+21*	+47	+15	+42
6	1	154	82	*+32*	+38	+27	+38
7	1	204	44	*+24*	+ 6	+11	−7
	2	216	37	*+36*	+ 6	+24	−5
8	1	162	82	*+29*	+ 9	+18	+14
9	1	137	136	*+28*	+39	+18	+ 6
	2	124	68	*+35*	+62	+35	+62
11	1	208	123	*+14*	−33	+14	−29
13	1	191	70	*+56*	+88	+54	+88

in the adrenal region. To clear up this point a final type of experiment was performed in which, besides exclusion of the hepatic factor by cutting the hepatic nerves, the vessels in the abdomen were tied as described by Cannon and Carrasco-Formiguera (6) so that the only way out from the adrenals into the general circulation was through the proximal end of the lumbo-adrenal veins into the shortened inferior cava. If, then, the abdominal cava was temporarily clamped above the lumbo-adrenal veins, only blood from the adrenals was at the moment prevented from joining the general circulation; and when the clamp was removed, only blood from the adrenals was allowed to reach the general circulation. This means that practically no hydrodynamic change in the circulation was produced either by clamping or unclamping the

abdominal cava. When the animal was thus prepared and the cava clamped, piqûre was performed and one minute thereafter the clamp was removed.

The main results of the two experiments of this type that were performed are summarized in table 4.[3] Portions of the record of experiment 14 are reproduced in figure 3 (lower record). It may be seen that while the cava is closed, so that no adrenal blood reaches the circulation,

TABLE 3

Changes in heart rate and blood pressure produced by faradic stimulation of the floor of the fourth ventricle

NUMBER OF EXPERIMENT	CONDITION ABOUT 10 SECONDS BEFORE PUNCTURE		MAXIMAL CHANGES WITHIN 1 MINUTE AFTER STIMULATION		REMARKS
	Heart rate	Blood pressure	Heart rate	Blood pressure	
6	120	45	*+45*	+65	Adrenal glands and vessels untouched
11	204	95	*+19*	+15, −24	Ibid.
10	139	68	− *4*	+23	Left adrenal out and right adrenal tied off

TABLE 4

Changes of heart rate and blood pressure produced by piqûre, while the adrenal blood is isolated from the general circulation and after removing the block

NUMBER OF EXPERIMENT	CONDITION ABOUT 10 SECONDS BEFORE BLOCK		MAXIMAL CHANGES AFTER PUNCTURE DURING BLOCK		MAXIMAL CHANGES WITHIN 2 MINUTES AFTER REMOVING BLOCK		CHANGES 2 MINUTES AFTER REMOVING BLOCK	
	Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure
12	138	65	−6	0	*+35*	+20	*+30*	+ 8
14	156	51	−6	+39	*+73*	+91	*+11*	+ 24

the heart rate, despite piqûre, goes down, probably due to absence of the epinephrin that is being constantly poured into the circulation under experimental conditions. When the cava is released one minute after the puncture so that the collected adrenal blood is allowed to enter the general circulation, a quick and remarkable increase of heart rate takes

[3] Experiment 13 was intended to be of this type but autopsy showed that wide, although indirect, connection between at least one adrenal gland and the general circulation remained; for that reason this experiment is practically one of the type described in section 2 and its results are therefore included in table 2.

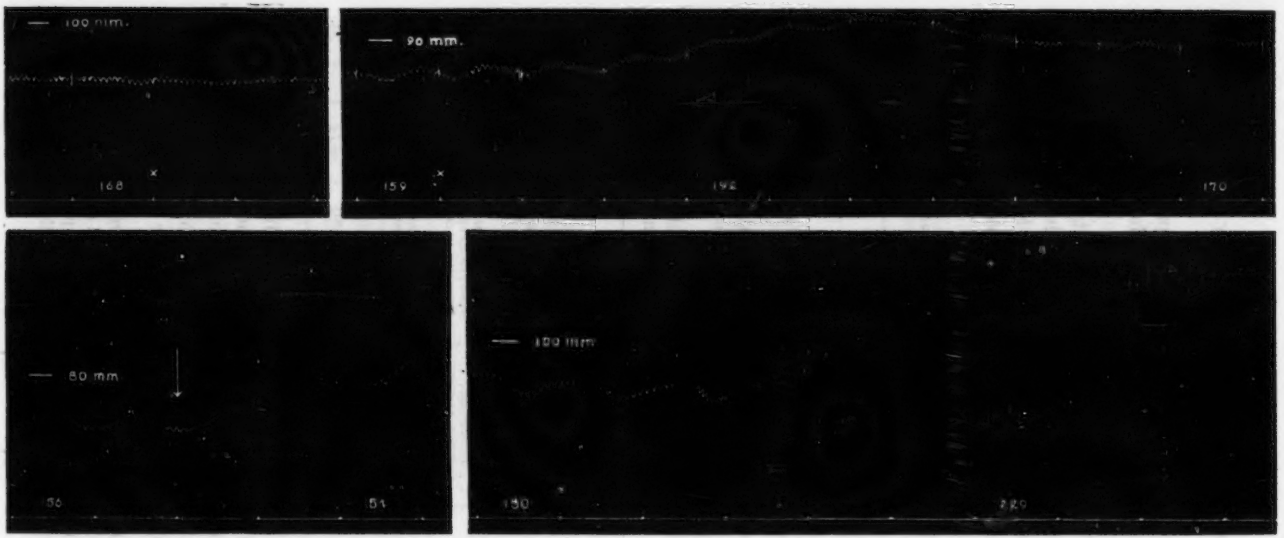


Fig. 3. Portions of records of beat of the denervated heart in an experiment in which (upper record) the adrenal blood was shut off from the circulation and readmitted one minute later without performing puncture, and (lower record) the adrenal blood was shut off from the circulation while puncture was performed and one minute after that it was released (no. 14, tables 4 and 5). The arrow marks the moment of puncture. The crosses mark the moments at which the adrenal blood was shut off from and readmitted to the circulation. In the upper record when no puncture was performed, before isolating the adrenal blood, the heart rate was 168; at the end of the block it was 159; 20 seconds after readmitting the adrenal blood it went up to a maximum of 192; 30 seconds later it was again 170.

In the lower record, when piqûre was performed, before excluding the adrenal blood the heart rate was 156; 15 seconds after the puncture, instead of being higher, it was 154; at the end of the period of exclusion, 50–60 seconds after the puncture, it was 150; 30 seconds after removing the block it was 229; 90 seconds later it was still 167.

The central portions of the records of the periods of exclusion of the adrenal block are omitted.

place. The rate goes up far above the original level and the acceleration lasts for at least more than 2 minutes.

Cannon and Carrasco-Formiguera (6) found that, under the conditions described, closing the cava for 45 seconds causes a steady fall in the heart rate and that, when the block is removed, no intentional stimulation having taken place, the heart rate returns to its original level. The restoration is sometimes preceded by a slight increase above the original level. In 5 experiments this increase averaged 6 beats per minute, with a maximal of 12 beats, and a duration of not more than some seconds. The slight increase seems to be attributable to concentration of epinephrin in the static blood and possibly also to slight local

TABLE 5

Changes in heart rate and blood pressure during and after block of the adrenal blood from general circulation without performing puncture or any intentional stimulation. (In experiment 14 the period of closure of the cava was 1 minute; in all the experiments by Cannon and Carrasco-Formiguera it was 45 seconds.)

CONDITION BEFORE BLOCK		MAXIMAL CHANGES DURING BLOCK		CHANGES WITHIN 1 MINUTE AFTER REMOVING BLOCK		CHANGES 1 MINUTE AFTER REMOVING BLOCK		NUMBER OR DATE OF EXPERIMENT
Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure	
168	67	−9	+3	*+24*	+33	*+2*	+7	14
192		−12		*+12*		*0*		January 24, C. and C.-F.
216		−18		*0*		*−6*		January 24, C. and C.-F.
216		−18		*+6*		*0*		January 24, C. and C.-F.
180		−10		*+12*		*−2*		February 7, C. and C.-F.
168		−12		*0*		*0*		February 8, C. and C.-F.

stimulation of the adrenals from clamping and unclamping of the cava. In experiment 14 a control test of this type was made before puncture; during the period of adrenal isolation (1 minute) the rate went down from 170 to 159 beats per minute; when the cava was released it rose to 192 and 50 seconds after opening the cava it was again 170. In this case it is almost certain that the movements of the clamp produced a slight stimulation of the adrenals, but even thus there is an enormous difference in intensity as well as in duration between the results of this control experiment and those obtained with the same animal when puncture was performed. The results of this control experiment, together with those obtained by Cannon and Carrasco-Formigura in analogous experiments, are given in table 5. Portions of the record of

this control test are reproduced in figure 3 (upper record). A comparison of table 5 with table 4 clearly shows that the great and long-lasting increases reported in table 4 must be due to something discharged from the adrenals at a greatly increased rate as a result of piqûre, because the cardiac acceleration obtained in these experiments does not occur so long as adrenal blood and nothing else, is prevented from reaching the coronary arteries. That this adrenal factor results from piqûre, and is not due to the presence and slight movements of the clamp in the abdomen or to anything else, is made clear by the fact that puncture was the only different feature of the experiments reported in table 4 as compared with those reported in table 5, for the presence and the slight movements of the clamp in the abdomen and every other experimental detail were common to both series of experiments.

As has been stated, Stewart and Rogoff (12) (43) claim that changes in denervated organs, such as those above mentioned in which splanchnic activity plays a part, may be accounted for without assuming any alteration in the rate of epinephrin output. They explain the effects either by improvement of the circulation in the denervated organs, or by some kind of "redistribution of blood" which results in an increase of flow and consequently of epinephrin in the denervated organ in unit time or in a greater concentration of epinephrin in that blood. What they call the "constant normal output of epinephrin" would be essential for the effectiveness of the "redistribution," and absence of this output would explain why isolation or extirpation of the adrenals prevents the usual effect. Cannon (4) and Cannon and Rapport (5) have proved, however, beyond doubt that these theoretical possibilities are not true for the denervated heart. Some of my results such as those of experiments 5 (puncture 1), 7 and 11, in which an increase of heart rate took place along with practically no change or with a decrease of blood pressure, are furthermore incompatible with Stewart and Rogoff's explanation.

With these objections removed, the results of the last described experiments, added to the evidence given by the preceding ones, can only be explained by admitting that the increased heart rate elicited by piqûre after the hepatic nerves are cut is due to something discharged from the adrenals into the blood, as a result of the puncture. If we admit that this "something from the adrenals," that has the power to accelerate the denervated heart, as adrenalin has, may be called epinephrin, beyond doubt puncture of the floor of the fourth ventricle produces a dis-

charge of epinephrin from the adrenals.[4] Adrenal discharge follows within a few seconds after puncture; in all of the cases the increase of heart rate lasted more than one minute; in two cases a record was taken 15 minutes after the puncture and the heart rate was still distinctly higher than the original level; in other cases a few minutes after piqûre the heart rate had come back to its original or a lower level. In the present work no attempt has been made to ascertain whether after this first discharge there are signs of any further increased output of epinephrin.

SUMMARY

1. A review of the literature shows that there is strong evidence in favor of the view that piqûre produces a discharge of epinephrin from the adrenals in sufficient amount to exert some action on other organs, but this evidence is not absolutely conclusive.

2. Bernard's puncture of the floor of the fourth ventricle increases the rate of the denervated heart.

3. Piqûre increases the heart rate even when the possible hepatic factor for this increase has been eliminated.

4. If after the possible hepatic factor has been excluded, the venous path from the adrenal glands is blocked in such manner that the only change at the moment is an exclusion of adrenal blood from the circulation, the increase of heart rate as a response to piqûre is prevented.

5. When the venous block is removed one minute after puncture the release of the adrenal blood causes a much greater and more lasting cardiac acceleration than if piqûre has not been performed during the block.

6. The increase of heart rate elicited by piqûre when the possible hepatic factor is excluded is due to nothing else than something discharged from the adrenals as a result of puncture.

[4] It should be clearly understood that because epinephrin is discharged by puncture, that does not necessarily mean that epinephrin plays any part in puncture hyperglycemia and glycosuria and still less that it is the only factor involved in those changes; and similarly because puncture hyperglycemia may be produced without the intervention of epinephrin, as seems to have been proved by Stewart and Rogoff (43), that does not necessarily mean that normally epinephrin plays no part in the production of the phenomenon.

CONCLUSION

Bernard's puncture of the floor of the fourth ventricle produces the discharge from the adrenal glands of a product that reaches the arterial blood in sufficient amount to exert on other organs an action identical to that of adrenalin.

BIBLIOGRAPHY

(1) Eckhard: Beitr. z. Anat. u. Physiol., 1869, iv, 3.
(2) Bernard: Lecons sur le Diabète, Paris, 1877, 371.
(3) Stewart, Rogoff and Gibson: Journ. Pharm. Exper. Therap., 1916, viii, 205.
(4) Cannon: This Journal, 1919, l, 399.
(5) Cannon and Rapport: Ibid., 1921, lviii, 308.
(6) Cannon and Carrasco-Formiguera: Ibid., 1922.
(7) Popielski: Arch. f. d. gesammt. Physiol., 1918, clxx, 245.
(8) Gley and Quinquaud: Journ. d. Physiol. et de Path. Gen., 1918, xvii, 807.
(9) Mayer: Compt. Rend. de la Soc. de Biol., 1906, lx, 1123.
(10) Kahn and Starkenstein: Arch. f. d. gesammt. Physiol., 1911, cxxxix, 181.
(11) Biberfeld: Arch. Exper. Path. u. Pharm., 1916, lxxx, 164.
(12) Stewart and Rogoff: This Journal, 1918, xlvi, 90.
(13) Jarisch: Arch. f. d. gesammt. Physiol., 1914, clviii, 478.
(14) Wertheimer and Battez: Arch. Internat. de Physiol., 1910, ix, 363.
(15) Freund and Marchand: Arch. Exper. Path. u. Pharm., 1914, lxxvi, 324.
(16) Kahn: Arch. f. d. gesammt. Physiol., 1911, cxl, 209.
(17) Kahn: Ibid., 1912, cxlvi, 578.
(18) Kahn: Ibid., 1917, clxix, 326.
(19) Starkenstein: Zeitschr. f. exper. Path. u. Therap., 1912, x, 78.
(20) Negrin: Arch. f. d. gesammt. Physiol., 1912, cxlv, 311.
(21) Negrin and V. Brücke: Treballs de la Soc. de Biol. de Barcelona, 1914, ii, 124.
(22) Borberg: Skand. Arch. d. Physiol., 1913, xxviii, 91.
(23) Sopeña: Boletin de la Soc. Española de Biolgia, 1918.
(24) Folin, Cannon and Denis: Journ. Biol. Chem. 1913, xiii, 477.
(25) Neubauer: Biochem. Zeitschr., 1912, xliii, 335.
(26) Trendelenburg and Fleischhauer: Zeitschr. f. exper. Med., 1913, i, 371.
(27) Negrin and Hernandez-Guerra: Boletin de la Soc. Española de Biologia, 1918.
(28) Cervera: Treballs de la Soc. de Biol. de Barcelona, 1917, v, 331.
(29) Watermann and Smit: Arch. f. d. gesammt. Physiol., 1908, cxxiv, 198.
(30) Ehrmann: Arch. f. exper. Path. u. Pharm., 1905, liii, 97.
(31) Cannon and de la Paz: This Journal, 1911, xxviii, 64.
(32) Trendelenburg: Arch. f. exper. Path. u. Pharm. 1910, lxiii, 161.
(33) Negrin: International Congress of Physiologists, Paris, 1920.
(34) Joseph and Meltzer: This Journal, 1912, xxix, p. xxxiv.
(35) Elliott: Journ. Physiol., 1912, xliv, 374.

(36) HOUSSAY ET CERVERA: Compt. Rend. de la Soc. de Biol., 1920, lxxxiii, 1281.
(37) ANREP: Journ. Physiol., 1912, xlv, 307, 318.
(38) HUNT: This Journal, 1899, ii, 444.
(39) LEVY: Heart, 1913, iv, 342.
(40) GASSER AND MEEK: This Journal, 1914, xxxiv, 63.
(41) CANNON: Science, 1917, xlv, 463.
(42) GLEY AND QUINQUAUD: Journ. de Physiol. et de Path. Gen., 1921, xix, 355.
(43) STEWART AND ROGOFF: This Journal, 1920, lii, 304.
(44) CANNON AND URIDIL: This Journal, 1921, lviii, 353.

THE ACTION OF ULTRA-VIOLET RAYS ON ARBACIA EGGS, ESPECIALLY AS AFFECTING THE RESPONSE TO HYPERTONIC SEA-WATER

RALPH S. LILLIE AND MARGARET L. BASKERVILL

From the Marine Biological Laboratory, Woods Hole, and the Nela Research Laboratories, Cleveland, Ohio

Received for publication May 1, 1922

The results of experiments in artificial parthenogenesis have shown that in the unfertilized egg, as in other irritable protoplasmic systems, a brief purely physical alteration may be the means of initiating the normal cycle of physiological activity, in this case cell-division and development. A control of metabolic, especially synthetic, processes by the structural conditions in the egg-system is thus indicated, and the more precise nature of this control remains to be determined. The detailed chemical nature of the sequence of events constituting activation is still largely unknown; in the sea-urchin egg a marked and rapid increase in oxidations, probably connected with the alteration of the surface-film, is the first observable change.[1] There is also evidence that chemical reactions involving the combination and disappearance of specific compounds contained in the egg-protoplasm occur within a few seconds after the entrance of a spermatozoon or the action of a parthenogenetic agent;[2] and it is to be presumed that the initiatory physical alteration acts directly or indirectly by promoting or facilitating such reactions, which form the first step in the specific sequence of reactions underlying and determining development.

It is remarkable that in certain eggs, notably the sea-urchin egg, the process of artificial activation seems never to be complete unless water is temporarily abstracted from the protoplasm, e.g., by hypertonic sea-water, during part of the activation-period, even although other conditions—presence of oxygen, temperature, composition of sea-water—remain normal. Apparently during this period of partial dehydration

[1] C. Shearer: Proc. Roy. Soc., 1922, xciii B, 213.

[2] F. R. Lillie: Journ. Exper. Zoöl., 1914, xvi, 523; *Problems of fertilization*, Univ. of Chicago Press, 1919, Chapters 5 to 7; Biol. Bull., 1921, xli, 125.

reactions are completed within the egg-system which in some way enable it to begin and carry through its normal cycle of development when returned to sea-water; in the absence of these reactions development ceases at an early stage.[3] In other echinoderm eggs, however, *e.g.*, the starfish egg, a complete activation can be induced by treatments which never effect more than a partial activation when applied to the sea-urchin egg, *viz.*, temporary exposure to fatty acid or high temperature; in this case all that is necessary is that the time of exposure to a definite concentration of acid or a definite temperature should be constant within somewhat narrow limits.[4] In the starfish egg, as well as in the sea-urchin egg, an incomplete activation caused by brief exposure to heat or fatty acid can be completed by after-treatment with hypertonic sea-water; but the same result can be obtained simply by prolonging or repeating the first or membrane-forming treatment.[5] This result shows that in the starfish egg the activating reaction (or complex of reactions) differs from that in the sea-urchin egg in not requiring a period of dehydration for its completion. Just why this latter condition should be necessary in the one egg but not in the other is a problem not yet solved; it seems probable that the ease with which development may be artificially induced in the starfish egg is connected with this peculiarity. There is the possibility that temporary lowering of the concentration of water in the egg-protoplasm may be favorable to certain dehydrolytic reactions which are of critical importance in the early metabolism of development;[6] this is an inference based on the general fact that the syntheses upon which organic growth depends (proteins from amino-acids, etc.) are mainly dehydrolytic in character. The general conditions suggest that the activating reaction (or reaction-sequence) occurs in two stages, both of which proceed to completion in the Asterias egg without any change in the total water-content of the system, but that in the Arbacia egg the second stage is completed only under conditions of relative lack of water in certain regions of the egg-system; this condition is attained more readily in artificial activation if the water-content of the egg as a whole is temporarily reduced. It is certain that the

[3] Compare Loeb's conclusion (*Artificial parthenogenesis and fertilization*, Univ. of Chicago Press, 1913, Chapter 12) that the "corrective" effect of hypertonic sea-water is due to the formation within the egg of some substance which acts as a preventive against the disintegration otherwise following membrane formation.

[4] R. S. Lillie: Biol. Bull., 1915, xxviii, 260; Journ. Biol. Chem., 1916, xxiv, 233.

[5] Biol. Bull., *loc. cit.*

[6] *Cf.* Biol. Bull., *loc. cit.*, p. 300.

physiological action of hypertonic sea-water depends primarily upon abstraction of water and not upon other possible effects produced by the solution—such as a change in the P.D. across the egg-surface—since hypertonic sea-water is equally effective when the osmotic pressure is increased without changing the concentration of electrolytes, *e.g.*, by adding sugar.

The responsiveness to treatment with hypertonic sea-water is found to vary widely in Arbacia eggs under both normal and artificial conditions. Different lots of normal eggs, treated with hypertonic sea-water alone for identical times and under apparently identical conditions, frequently yield very different proportions of swimming blastulae; in some cases a relatively brief, in others a longer exposure is required for the activation of a significant proportion of eggs; and usually the great majority break down in early development. Whether the individual egg exhibits normally a regular cycle of susceptibility to this treatment does not seem to have been determined; the existence of this variability is, however, in itself a fact of interest in relation to the problem of the physiological conditions underlying activation. Artificial treatments of various kinds also change the responsiveness to hypertonic sea-water. There is always a much greater response if the eggs are first treated with a membrane-forming agent, as Loeb first showed;[7] in Arbacia the effective duration of exposure is reduced to one-third or one-fourth of that required without previous membrane-formation, and the proportion of eggs developing to advanced stages is greatly increased. Evidently the process which occurs in the egg during the partially dehydrated condition is in some way facilitated by the membrane-forming treatment; and since the immediate effect produced by this treatment appears to be superficial, consisting apparently in a temporary breakdown or de-emulsification of the surface-layer of the egg, with increase of permeability and probably change of electrical polarization as consequences, we may infer that the critical reaction (or reaction-sequence) induced by hypertonic sea-water is also one occurring within the surface-layer. This inference is in harmony with Chambers' recent observations showing that the surface layer (cortical zone) of the egg is the seat of the primary fertilization-reaction, and that no reaction is shown by isolated portions of the internal protoplasm (endoplasm).[8]

The susceptibility of Arbacia eggs to hypertonic sea-water may also be increased under conditions which do not involve membrane-forma-

[7] J. Loeb: Univ. of California Publications, Physiology, 1905, ii, 83.

[8] R. Chambers: Journ. Gen. Physiol., 1921, iv., 49; Biol. Bull., 1922, xli, 318.

tion or other visible change in the egg. This occurs, for example, when the eggs are placed for a brief period in an unbalanced isotonic salt solution (*e.g.*, pure NaCl); such eggs when returned to sea-water remain apparently unchanged for 24 hours or longer, and if fertilized during this period develop normally. It is found, however, that if they are treated with hypertonic sea-water, at any time during this period, they react in essentially the same manner as eggs that have been treated a short time previously with a membrane-forming agent; a considerable proportion develop to the larval stages after relatively brief exposures. The change in the reaction to hypertonic sea-water is similar to that following membrane-formation, although as a rule it is less pronounced; but the eggs differ from those with membranes in remaining alive, fertilizable, and in other respects apparently unaltered for 24 hours or longer after treatment.[9] A similar effect may be produced by brief exposure to ultra-violet light, as well as by mechanical treatment and high temperature, as will be shown below.

Since the essential change produced by the unbalanced salt solution is apparently an increase in the permeability of the surface-layer of the egg, and since the same kind of change is produced by cytolytic or membrane-forming agents, we may infer that this increase of permeability is a condition favorable to the formation of some compound (or compounds) determining the susceptibility to hypertonic sea-water. It may be that the substances uniting to produce this sensitizing compound diffuse more readily during the period of increased permeability; or the effect may be a direct result of the altered electrical polarization of the cell-surface, as in stimulation-processes generally. Apparently, as a second stage in the reaction-sequence of activation, the compound thus formed requires to be built up by dehydrolytic synthesis into some further combination, and this occurs most readily in the sea-urchin egg if the water-content is temporarily reduced.

In general, since development is a matter of growth, *i.e.*, of specific synthesis, it would seem probable that the essential conditions at the beginning of development must be those which initiate and promote synthetic reactions within the egg; the increase in oxygen-consumption is probably thus to be explained, since oxygen is necessary for most growth processes; this consideration may also explain why oxygen is necessary for the action of hypertonic sea-water.[10]

[9] R. S. Lillie and M. L. Baskervill: This Journal, 1921, lvii, 110.

[10] J. Loeb: *Artificial parthenogenesis and fertilization*, Chapter 11.

Experimental: Direct action of ultra-violet rays on unfertilized eggs. The eggs were exposed to the rays from the mercury arc in flat-bottomed vessels in a shallow layer of sea-water, the lamp used and the conditions of experimentation being the same as those described in our recent paper on starfish eggs.[11] The immediate effects produced by the rays—local injury, formation of membranes, cytolysis—are in general similar to those in starfish eggs, but local cytolyzed areas are less frequent, and complete fertilization-membranes are more readily formed; the eggs are also more resistant to injury; thus 15 minutes' exposure to the arc at 14 cm. proved fatal to all starfish eggs, but with Arbacia almost twice this exposure was required to produce a corresponding degree of injury. This is illustrated by the following experiment (table 1).

TABLE 1

June 14. The eggs were exposed in a shallow layer of sea-water to the mercury arc at 14 cm. distance, and at the intervals indicated portions were placed in a series of vessels containing sea-water; all eggs were fertilized at the same time, about 35 minutes after the first exposure to the rays. The approximate proportions forming blastulae are given.

Duration of radiation (minutes)	1	2	4	7	10	15	20	30
Per cent blastulae	100	95	75	60–70	60–70	40–50	10–15	0

In a second similar experiment with the arc 25 cm. from the eggs about one-third formed blastulae when fertilized after 30 minutes' exposure.

Apparently it is characteristic of the action of radiation on living matter (as well as on the photographic plate) to produce latent effects which manifest themselves afterwards in the altered chemical behavior of the system. This is well seen in the effects of brief radiation on unfertilized eggs. In general eggs exposed for 1 to 2 minutes to radiation of the above intensity (14 cm. from arc) and left undisturbed in sea-water show no immediate alteration and with few exceptions remain normal and fertilizable for a day or longer; 3 minutes' radiation is usually followed within the next 24 hours by the spontaneous breakdown of a small proportion of eggs. This tendency to spontaneous cytolysis increases rapidly with further duration of radiation; it also varies considerably in different lots of eggs; for example, in one instance eggs radiated for 1 and 2 minutes showed almost no cytolysis next day; after an exposure of 7 minutes about 10 per cent and after one of 10 minutes about 50 per cent underwent cytolysis in the same time. In another

[11] R. S. Lillie and M. L. Baskervill: This Journal, 1922, lxi, 57.

experiment the cytolysis following 3 minutes' exposure was 10 to 15 per cent and after 5 minutes, exposure about 50 per cent. A similar initiation of progressive changes culminating in cytolysis may also be produced by other forms of treatment which apparently have no immediate effect upon the eggs, *e.g.*, exposure to isotonic salt solutions, moderate shaking, or treatment with alcohol-containing sea-water.

The increased tendency to cytolysis in radiated eggs is apparently an indication of structural changes produced in the surface-layer of the protoplasm by the rays; this effect is associated with an activating influence more or less definitely expressed.[12] Eggs examined within 3 to 5 hours after a radiation lasting 3 minutes or less show with few exceptions no visible fertilization-membranes or other change; after exposures lasting 5 to 10 minutes a visible separation of membranes is usually observed in a considerable proportion of eggs, although much variation is seen. In some lots of eggs longer exposures up to 15 minutes produced no visible membranes; later, however, these eggs underwent changes similar to those exhibited by eggs with separated membranes. It is, however, a well-known peculiarity of Arbacia eggs to vary widely in the degree of membrane-separation caused by artificial treatment.

Irregular change of form and cleavage (usually slow and irregular) typically follow in a variable proportion of eggs within 2 or 3 hours after a radiation lasting 5 minutes or longer. In most eggs cleavage does not proceed far and is followed by cytolysis, but in some experiments an occasional egg developed to the blastula stage without any further treatment; a similar result has been observed in eggs treated temporarily with isotonic NaI or KCNS solutions.

The tendency of radiated eggs to break down spontaneously within a few hours indicates that the rays cause physical changes in the egg system similar to those resulting from the action of other membrane-forming agents or isotonic salt solutions. An increased responsiveness to hypertonic sea-water appears to be an invariable consequence of such changes and this effect is well-marked in radiated eggs;[12] if the latter are exposed soon after radiation to hypertonic sea-water for 20 to 40 minutes (at 20°) a greatly increased proportion cleave (instead of simply undergoing irregular change of form and breakdown) and of these many may develop to the blastula stage. Tables 2 and 3 give a summary of typical experiments illustrating the parthenogenetic action of exposure for varying times to radiation followed by treatment with hypertonic sea-water. In these experiments the eggs were exposed at a distance of 14 cm. from

[12] J. Loeb: Science, 1914, xl, 680.

the lamp for the times indicated, and after a brief interval were treated with hypertonic sea-water and returned to normal sea-water; the proportion of eggs developing to the blastula stage was determined next day.

Table 2 gives the results of four typical experiments which included longer exposures up to 15 minutes. It will be noted that except in experiment 2 (in which the eggs were exceptionally responsive to hypertonic sea-water) the sensitizing effect of 1 to 2 minutes' radiation is comparatively slight; with 4 to 10 minutes' exposure sensitization is well marked, while one of 15 minutes is above the optimum. This result corresponds with the fact already cited (table 1) that 15 minutes' radiation renders most eggs incapable of development beyond early stages.

An appreciable sensitizing effect is produced by exposures so brief as 1 minute, although in other respects this treatment leaves the eggs apparently unaltered. As already described, there is usually no separation of membranes with a radiation of 4 minutes or less. Visible separation of membranes means, however, merely that the initial part of the activation-process has reached a certain stage. Until this stage is reached the general properties of the egg are apparently unchanged. The results of table 1 show that eggs radiated for 1 to 2 minutes respond almost normally to sperm-fertilization and that a distinctly injurious effect does not begin to appear until radiation has lasted 4 minutes or longer. What is chiefly interesting is that eggs which show no visible alteration, and develop normally on fertilization, show nevertheless a distinct increase in their responsiveness to hypertonic sea-water. A similar result may be obtained by brief treatment with isotonic salt-solutions and other agents (see below); it may be regarded as indicating an incipient activating effect, although insufficient for membrane-formation or other visible alteration.

In a later series of 10 experiments the effects of brief exposures were examined more closely. In most cases a slight but distinct increase of susceptibility was found after exposure to the above radiation (14 cm.) for one minute; with exposures of 2 minutes the sensitizing effect was more definite, and with exposures of 3 to 5 minutes, well marked. Table 3 summarizes the results of four typical experiments on four consecutive days. It will be noted that the responsiveness induced by radiation of a given duration varies considerably in different lots of eggs; in general the best results were obtained with 4 to 5 minutes' radiation; on September 5 the eggs were less responsive than on the other days.

It is somewhat remarkable that the increased susceptibility induced by brief ultra-violet radiation largely disappears during the 24 hours

TABLE 2

NUMBER AND DATE OF EXPERIMENT	DURATION OF RADIATION (MINUTES)	APPROXIMATE PERCENTAGE OF EGGS FORMING BLASTULAE AFTER TREATMENT WITH HYPERTONIC SEA-WATER (100 VOLS. SEA-WATER + 20 VOLS. 2.5 M NaCl) FOR THE TIMES INDICATED			
		20 minutes	30 minutes	40 minutes	60 minutes
1. June 16	1	0	0	<1%	
	2	0	0	<1	
	4	<1	<1%	35–40	
	7	<1	2–3%	*ca.*25	
	10	0	0	*ca.* 25	
	15	0	0	<1	
	0 unradiated (control)	0	0	<1	
2. June 18	1		15–20	few <1	<1
	2		20–25	2–3	<1
	4		25–30	2–3	1–2
	7		*ca.* 10	2–3	<1
	10		*ca.* 10	5–10	<1
	15		5–10	*ca.* 10	<1
	0 (control)		2–5	*ca.* 5	<1
3. June 24	1	0	0	0	
	2	0	<1	*ca.* 1	
	4	*ca.* 5	1–2	<1	
	6	*ca.* 10	*ca.* 20	2–3	
	8	<1	5–10	*ca.* 5	
	10	0	2–3	1–2	
	15	<1	0	0	
4. August 26	1	<1	<1	*ca.* 1	
	2	<1	5–10	2–3	
	3	*ca.* 1	*ca.* 10	*ca.* 5	
	4	2–3	*ca.* 10	*ca.* 10	
	5	5	10–15	*ca.* 10	
	6	2–3	10–15	*ca.* 10	
	8	2–3	10–15	5–10	
	0 (control)	0	<1	<1	

succeeding, although in other respects the eggs may show little evidence of change. In eight experiments in which eggs radiated for periods of

1 to 4½ minutes were left undisturbed in sea-water for 24 hours before after-treatment, the response to hypertonic sea-water was in every case much less than in eggs from the same lots which received the hypertonic treatment immediately after radiation; this decline was not due to the death of the eggs, for many remained intact and when fertilized developed to the blastula stage. In five out of the eight experiments, however, the radiated eggs yielded a larger proportion of blastulae as a result of the treatment with hypertonic sea-water than the unradiated

TABLE 3

NUMBER AND DATE OF EXPERIMENT	DURATION OF RADIATION	TIME IN HYPERTONIC SEA-WATER AND PERCENTAGE OF EGGS FORMING BLASTULAE			
		30 minutes	45 minutes	60 minutes	90 minutes
6. September 5	1.5 min.	0	0	0	<1
	2.5 min.	0	<1	<1	<1
	3.5 min.	<1	*ca.* 1	1–2	1–2
	0 (control)	0	<1	<1	<1
8. September 6	2.5 min.	<1	<1	*ca.* 1	2–3
	3.5 min.	<1	1–2	5–10	*ca.* 10
	4.5 min.	<1	*ca.* 5	*ca.* 25	25–35
	0 (control)	0	0	0	<1
9. September 7	2 min.	<1	<1	<1	<1
	3 min.	1–2	1–2	4–5	2–3
	4 min.	1–2	1–2	4–5	*ca.* 5
	0 (control)	0	0	0	<1
10. September 8	3 min.	*ca.* 1	1–2	1–2	2–3
	4 min.	3–4	3–4	<1	<1
	5 min.	*ca.* 10	*ca.* 5	*ca.* 1	<1
	0 (control)	0	0	0	0

eggs had given on the previous day; the hyper-susceptibility thus remains in part, although greatly reduced. Evidently the sensitization resulting from radiation is of a less permanent kind than that induced by isotonic salt-solutions; the latter remains almost unchanged for 24 hours or longer.[13]

This progressive decline in responsiveness in eggs sensitized by radiation is probably to be correlated with the increased tendency to spontaneous cytolysis or decrease in the length of life in sea-water, already

[13] R. S. Lillie and M. L. Baskervill: This Journal, 1921, *loc. cit.*, 120.

described. Apparently some injurious or irreversible effect is associated with the action of the rays. This effect exhibits itself in a general decline in fertilizability and developmental power; *e.g.*, in a series in which eggs were radiated for periods varying from 1 to 8 minutes and fertilized 24 hours later, the proportion forming blastulae decreased progressively with increase in the duration of radiation, from 90 per cent or more in the eggs radiated 1 and 2 minutes to about 25 per cent in those radiated 8 minutes.

Apparently the increase in susceptibility to breakdown or spontaneous cytolysis has the same physical basis as the increase in responsiveness to hypertonic sea-water; and both effects are almost certainly to be referred to changes induced in the surface-layer of the eggs by the rays. This is further indicated by the general nature of the effects following the exposure of radiated eggs to hypertonic sea-water. These effects vary greatly in their visible character; in many eggs changes are produced which have little resemblance to normal activation and lead to rapid cytolysis, while in others more or less regular cleavage is initiated, followed by development to advanced stages. Even under the most favorable conditions, however, the majority of radiated eggs after-treated with hypertonic sea-water simply undergo irregular changes of form, or if cleaving, break down before reaching the blastula stage; in general the proportion thus affected increases both with the duration of radiation and with the time of exposure to hypertonic sea-water. In table 4 a record is given of a series of experiments illustrating the parallelism of these effects.

Evidently the activating effect and the cytolysis-inducing effect of the hypertonic treatment run closely parallel. This general fact appears to throw light on the nature of the physical change produced by the rays. The preliminary alteration which renders the eggs more susceptible to the hypertonic treatment is, as already shown, not necessarily a cytolytic one (in the sense of causing irreversible breakdown of cell-structure), but is better described as being of a kind which, *if it proceeds far enough*, may lead to cytolysis. Even if insufficient in itself to produce this effect, it increases the predisposition to cytolytic alteration as well as to activation. The evidence from this and other experimental data in this field points clearly to a change in the protoplasmic surface-layer (plasma-membrane or cortical zone)—presumably involving alteration or breakdown of the normal film-structure in this region of the egg—as the probable basis of this change in properties.

TABLE 4

August 29, 1921. Eggs were exposed in a layer of sea-water 0.5 cm. deep to rays from the Hg-arc at a distance of 15 cm. and transferred to dishes of sea-water after exposures of 1,2 and 3 minutes. Eggs of each radiated lot and a control of unradiated eggs were after-treated with hypertonic sea-water (250 cc. sea-water plus 50 cc. 2.5 m. NaCl) in the usual way for periods of 20, 30, 40, 60, 90 and 120 minutes. The condition of the eggs in the different dishes 20 hours later was as follows.

TIME IN HYPERTONIC SEA-WATER	DURATION OF EXPOSURE TO RAYS			
	0 (control)	1 minute	2 minutes	3 minutes
0 (control)	Eggs unchanged	Eggs almost unchanged; a few cytolyzed	Little change; a few cytolyzed (<5 per cent)	Most eggs unchanged; 5–10 per cent cytolyzed; no blastulae
20 min.	A few blastulae; *ca.* 10 per cent cytolyzed eggs; rest unchanged	<1 per cent blastulae; *ca.* 15 per cent cytolyzed	2–3 per cent blastulae; 75 per cent cytolyzed	2–3 per cent blastulae; 90 per cent cytolyzed
30 min.	No blastulae; *ca.* one-third cytolyzed	<1 per cent blastulae; 30–40 per cent cytolyzed	10–15 per cent blastulae; 90 per cent of remaining eggs cytolyzed	10–15 per cent blastulae; almost all remaining eggs cytolyzed
40 min.	<1 per cent blastulae; *ca.* 75 per cent cytolyzed	<1 per cent blastulae; *ca.* 80 per cent cytolyzed	1–2 per cent blastulae; 90 per cent of remaining eggs cytolyzed	*ca.* 10 per cent blastulae; almost all remaining eggs cytolyzed
60 min.	<1 per cent blastulae; *ca.* 90 per cent cytolyzed	<1 per cent blastulae; all remaining eggs cytolyzed	1–2 per cent blastulae; all remaining eggs cytolyzed	2–3 per cent blastulae; all remaining eggs cytolyzed

None of the radiated eggs exposed to hypertonic sea-water for 90 and 120 minutes formed blastulæ, and all were cytolyzed next day.

If such treatments as brief radiation and exposure to isotonic salt-solutions can, without visibly altering the egg, increase its responsiveness to hypertonic sea-water, and if the basis of this effect is some structural change in the protoplasmic surface-layer, it should be possible to produce the same type of sensitization by other forms of treatment which, though not ordinarily forming fertilization-membranes, may be presumed also to act upon the surface-layer. It has been shown in one of our former papers that exposure to isotonic salt solutions has this effect. During the past summer we have experimented with the following treatments: *a*, mechanical agitation; *b*, exposure to weak solutions of alcohol in sea-water; *c*, exposure to high temperatures (32° to 34°). In the starfish egg all of these treatments have an activating or activation-promoting influence under appropriate conditions, but they are without evident action of this kind upon the Arbacia egg.

Mechanical agitation. Unfertilized eggs were shaken by hand in test tubes or glass-stoppered cylinders for periods varying from a few seconds to several minutes; the shaking was uniform and moderate so that its direct destructive action was gradual; after an interval of some minutes in normal sea-water the shaken eggs were treated with hypertonic sea-water in the usual manner. Table 5 gives the results of a typical experiment.

The results of this and similar experiments show considerable irregularity, but certain effects are definite and constant; in all cases shaking increases the proportion of eggs which undergo membrane-formation, cleavage and incipient development as a result of brief after-treatment with hypertonic sea-water. The degree of the sensitizing effect (with moderate shaking) increases as the shaking is prolonged up to 2 or 3 minutes; with longer shaking the direct mechanical injury becomes too severe and few eggs develop. Six experiments similar to the above were performed with results which, although varying in detail, were concordant in their main features. The proportion of eggs developing to larval stages was always low (15 to 20 per cent was the maximum), the majority merely forming membranes and undergoing cytolysis, usually preceded by irregular cleavage or change of form. Unshaken eggs required much longer exposure to hypertonic sea-water to cause the same degree of cytolytic alteration or activation. In the qualitative sense the results of this treatment resemble closely those of brief radiation.

Alcohol-containing sea-water and high temperature. The results of experiments with alcohol-containing sea-water and high temperature

TABLE 5

July 5, 1921. Eggs were shaken by hand in a test tube about half filled with a suspension of eggs in sea-water for the times indicated, transferred to dishes of sea-water, and shortly afterwards treated with hypertonic sea-water for periods of 20, 30 and 40 minutes. The condition of the eggs 3 hours after the treatment and next day (24 hours) was as follows (observations refer to proportions cleaving, forming blastulæ, and breaking down in early development or without development):

TIME IN HYPERTONIC SEA-WATER	DURATION OF SHAKING					
	0 (control)	A few seconds (4 shakes)	½ minute	1 minute	2 minutes	3 minutes
0	No change	No change	10 per cent cytolysis; no cleavage or blastulae	⅓ to ½ cytolysis; no blastulae	*ca.* 50 per cent cytolysis; no blastulae	*ca.* 75 per cent cytolyzed; no blastulae
20 min.	*ca.* 20 per cent cytolysis; *ca.* 80 per cent unchanged; a few blastulae	Some defective cleavage; *ca.* 50 per cent cytolyzed; no blastulae	Slight cleavage; *ca.* 90 per cent cytolyzed; no blastulae	90 per cent cytolyzed; no blastulae	90 per cent cytolyzed; no blastulae	90 per cent cytolyzed; a few blastulae
30 min.	1–2 per cent cleavage; 50 per cent cytolyzed; no blastulae	*ca.* 10 per cent irregular cleavage; 75 per cent cytolyzed; <1 per cent blastulae	*ca.* 10 per cent irregular cleavage; 90 per cent cytolyzed; *ca.* 1 per cent blastulae	*ca.* 10 per cent irregular cleavage; 90 per cent cytolyzed; *ca.* 1 per cent blastulae	*ca.* 10 per cent irregular cleavage; 90 per cent cytolyzed; 1–2 per cent blastulae	*ca.* 1–2 per cent blastulae
40 min.	*ca.* 20 per cent cleavage; 50 per cent cytolyzed; *ca.* 5 per cent blastulae	*ca.* 25 per cent irregular cleavage; *ca.* 1 per cent blastulae	30–40 per cent cleavage; 90 per cent cytolyzed; *ca.* 5 per cent blastulae	*ca.* ½ cleaved irregularly; 90 per cent cytolyzed; ca. 1 per cent blastulae	*ca.* ½ cleaved; 90 per cent cytolyzed; 5 per cent blastulae	*ca.* 50 per cent cleaved; 15–20 per cent blastulae; rest cytolyzed

were similar, but the proportion of eggs developing to larval stages was smaller. A brief summary of these experiments is sufficient. Eggs were exposed to sea-water containing ethyl alcohol (from 1 to 6 volumes per cent) for periods varying from 10 to 30 minutes, then transferred to normal sea-water and treated with hypertonic sea-water as usual. Eggs treated with alcohol alone show little change, but when after-treated with hypertonic sea-water, they respond in the same manner as shaken or slightly radiated eggs; *i.e.*, an increased proportion show cleavage or cytolytic alteration, and in favorable cases form blastulae. In most experiments the development was poor; the best results were obtained with exposures of 10 to 20 minutes and 2 to 5 per cent alcohol. Experiments with high temperature gave in general similar results; the eggs were exposed to warm sea-water (32° and 34°) in beakers, for periods varying from 2 to 10 minutes, returned to normal sea-water, and after-treated with hypertonic sea-water as usual.

General discussion. The above experiments indicate that brief radiation with ultra-violet rays produces in unfertilized eggs effects similar in kind to those caused by mechanical treatment, exposure to lipoid-alterant compounds or high temperature, or the action of unbalanced salt solutions. All of these agents appear to act by producing structural changes in the egg-system, primarily in the surface layer; and as a secondary physiological consequence the responsiveness of the egg to hypertonic sea-water is increased. There appears to be nothing specific in the effects produced by radiation. The energy of the absorbed rays is locally transformed, and the resulting structural and other changes in the protoplasmic system are similar to those produced by other physical agents and have similar physiological effects.

Apparently the basis of the sensitizing effect is some physical change of a kind to which the peculiar structural constitution of living protoplasm renders it especially liable; the nature of this change seems largely independent of the special physical nature of the agent, as in cases of "releasing" action in general. The common feature in the action of all of the above agents is the production of irreversible structural breakdown or cytolysis if the action is long-continued; it may be assumed that with brief action the physical changes which they cause in the protoplasm are of the same kind as those determining cytolysis, but differ in degree, being relatively slight or reversible. Hence the sensitized egg may show no external signs of alteration (as with brief radiation or isotonic NaCl); while in those cases where its structure is visibly altered (as in membrane-formation), the change does not

proceed far enough to prevent recovery under appropriate conditions. The effect of even a brief structural change of the kind assumed is, however, to change the reaction of the egg-system to hypertonic sea-water in the definite manner described.

Just why a slight and temporary structural alteration should so modify the physiological properties and metabolism of the egg cannot be said in detail at present. The general problem to be first solved is the problem of the relation of protoplasmic structure to the reactivity and metabolism of the living cell. A more precise knowledge of the physical nature of this structure is required. We have already much direct evidence, however, that the chemical reactions in protoplasm are under the control of structural conditions. Cytolytic agents change not only the physical structure of protoplasm but also the rate and character of its chemical reactions; many examples of such effects are known (increased oxidations, formation of acid, specific enzyme and other reactions). On the purely physical side the most evident effect produced by cytolytic agents is a destruction of the semi-permeable or diffusion-hindering property of the protoplasmic partitions; these partitions include not only the external surface-layer or plasma-membrane, but apparently also an internal system of membranes or films, or intracellular film-structure. The chemical effects of cytolysis indicate that changes in these partitions determine or release many reactions which do not occur (or occur only slowly) while the normal or resting protoplasmic structure remains unaltered. There is also much evidence from the physiology of stimulation that under normal conditions the reactions occurring in the irritable system are controlled by variations in the protoplasmic film-structure.[14]

With these general facts and considerations as a basis we may perhaps form a clearer conception of the elementary physical processes determining the activation and development of the egg-cell. The indications from the related field of the physiology of stimulation are that changes in the permeability, electrical polarization and other properties of the protoplasmic partitions (plasma-membranes, alveolar walls, surface-films of fibrils, etc.) control the chemical reactions and hence the energy-production of irritable cells. In the activation of the egg-cell there is every indication that similar factors are concerned; and what is true of the initiatory stages of development is probably true also of its later stages. We may infer, therefore, since development is essentially

[14] *Cf.* the recent review of the physiology of stimulation and transmission by R. S. Lillie in Physiological Reviews, 1922, ii, 1; *cf.* pp. 22 *seq.*

a matter of metabolic and structural synthesis, that changes in protoplasmic film-structure are among the main factors controlling the synthesis of specific compounds, not only at the beginning of development but through all of its stages.

The general physical structure of protoplasm is consistent with this view; there seems now to be general agreement that this structure is emulsion-like; *i.e.*, that the protoplasmic system is built up of phases of different composition which are separated from one another by thin surface-films. Protoplasm seems, however, to have special peculiarities of structure which differ in certain fundamental respects from those found in most artificial types of emulsion. The indications are: *a*, that in many diphasic forms of protoplasmic emulsion-structure the same continuous medium or solvent, water, is present in *both* phases of the system;[15] in this respect such a system would resemble other biological systems larger in scale, such as a suspension of blood corpuscles in serum; also *b*, that the films separating these phases are chemically unstable, especially in irritable forms of protoplasm, and subject to alternating processes of breakdown and reformation, occurring either spontaneously or under the influence of stimulating agents. Such a type of structure implies that free diffusive communication may be temporarily or intermittently established between adjacent protoplasmic phases as a result of stimulation or activation; at such times interaction may occur between compounds which at other times are kept separate. If, as seems probable from the facts already cited, the rate, character and sequence of the metabolic reactions of activation and development are largely regulated by such means, it is clear that artificial modification of protoplasmic structure would also influence these reactions; the effects produced by sensitizing agents would then fall within this general category.

Such a conception again emphasizes the closeness of the relations existing between the synthetic or formative processes of protoplasm and stimulation-processes in general, in the latter of which we have clear evidence that breakdown and reformation of film-structure are essential factors.[14]

[15] For example, the secretion-containing alveoli of gland cells (or of such eggs as the Nereis egg) and the interstitial protoplasm represent two such phases. Similarily we may regard the fibrils of a muscle cell and the sarcoplasm as separate phases; obviously in protoplasm, as well as in many other colloidal systems, the surfaces of separation of the phases are not necessarily spherical.

SUMMARY

1. Exposure of unfertilized Arbacia eggs to ultra-violet radiation (mercury arc of *ca.* 2 amperes, 110 volts, at 14 cm. distance) for 5 to 15 minutes causes membrane-formation, associated with cytolytic action. Eggs in which membranes are thus formed show (unless over-exposed) the usual increased susceptibility to the activating influence of hypertonic sea-water.

2. In general this increase of susceptibility in radiated eggs runs parallel (up to exposures of about 7 or 8 minutes) with an increased tendency to cytolytic breakdown, shown both in eggs left in sea-water without further treatment and in eggs after-treated with hypertonic sea-water.

3. Radiation too brief to produce membranes or evident cytolytic alteration (1 to 3 minutes) also increases the responsiveness to hypertonic sea-water. A similar effect is produced by mechanical treatment (shaking), high temperature (32° to 34°), and certain forms of chemical treatment (isotonic neutral salt solution, weak solutions of alcohol in sea-water).

4. It is suggested that the common factor in the action of these various agents is a change in the film-structure of the surface-layer of the egg-protoplasm; apparently this structure is temporarily broken down more or less completely by activating or sensitizing agents as well as in normal fertilization; this change permits the interaction of specific substances which in the unactivated egg are kept apart.

THE INFLUENCE OF HYDROGEN SULPHIDE UPON RESPIRATION

HOWARD W. HAGGARD AND YANDELL HENDERSON

WITH THE COLLABORATION OF T. J. CHARLTON

Investigations performed for the U. S. Bureau of Mines in the Laboratory of Applied Physiology, Yale University

Received for publication May 1, 1922

The body has the capacity to handle H_2S up to a certain limit entirely without ill effect. This is extraordinary, for a slightly larger amount is quickly fatal. Thus the effects upon respiration and other functions vary widely as between low, medium and high concentrations. These variations are, we believe, very significant for the correct interpretation of the reactions between the living body and this substance. It is an acute poison, comparable to cyanide, the effects of which are indeed quite similar (1), and yet it is frequently formed in considerable amount in the intestines so that perceptible quantities—corresponding to the concentration in the blood—may be excreted in the breath of persons who are virtually healthy.

The inhalation of an atmosphere of the minimal lethal concentration of H_2S (approximately 5 volumes of H_2S in 10,000 volumes of air for dogs) causes death only after the lapse of many hours of continued exposure and is then apparently due to lung edema. With this concentration the respiratory effects are not very marked: a slight progressive depression of the respiration, both of the rate and apparently also of the depth, is observed.

With double this concentration of H_2S (10 of H_2S in 10,000 of air or 0.1 per cent) in the inhaled air, death occurs in 15 to 20 minutes. Almost from the first respiration is markedly augmented; a violent hyperpnea develops and terminates in 10 or 15 minutes in an apnea vera during which the animal dies.

With still higher concentrations (30 parts of H_2S in 10,000 of air, 0.3 per cent, or over) the effect is fulminating. After a few violent gasps respiration is suddenly paralyzed.

Exactly the same effects result from intramuscular or intravenous injection of sodium sulphide; for, as has been shown in a recent paper from this laboratory (2), Na_2S is immediately and practically completely hydrolyzed in the blood with the liberation of H_2S. The effects produced by the injection of sodium sulphide are wholly due to this liberated H_2S and thus are identical with those of the gas.

In the paper just referred to, it has also been shown that H_2S introduced into the blood stream, either through the inhalation of this gas or by the hydrolysis of sodium sulphide in the blood, is rapidly oxidized to non-toxic compounds. The explanation of the capacity of the body to withstand small amounts of H_2S, while slightly more is markedly toxic, evidently depends upon this capacity of the blood to oxidize and thus detoxicate H_2S up to a certain limit.

It has also been shown in that paper that no combination of H_2S is effected with the hemoglobin, except under diseased conditions of special etiology. The effects of H_2S are, therefore, not in the nature of an asphyxia, except to the very slight extent to which the blood is depleted of oxygen in the oxidation of the H_2S. The physiological effects of H_2S are exercised through the gas in solution in the plasma of the blood.

The respiratory response to sulphide must then be a result of the action of the H_2S dissolved in the blood upon some mechanism controlling respiration. There are three possibilities: *a*, The action might be directly and specifically upon the respiratory center in the medulla; *b*, it might alter the hydrogen ion equilibrium of the blood; *c*, it might stimulate some respiratory mechanism other than the center, as for example, the vagal endings in the lungs.

The points of action of sulphide. For the investigation of these three possibilities the action of sulphide was observed after section of the vagi. It has been shown by Scott (3) that a respiratory response is obtained from the inhalation of CO_2 even after the vagi have been cut. Thus, by analogy, if H_2S also acts directly upon the respiratory center or alters in significant amount the C_H of the blood, section of the vagi should not prevent its action in augmenting breathing.

Our experiments show, however, that after section of the vagi injections of sodium sulphide in moderate amounts do not augment respiration. The stimulating action of moderate doses is apparently exerted upon the vagal endings in the lungs. The action of high concentrations upon the center is generally depressant. The same is true of inhalations of H_2S as observed in other experiments. In only one of many

experiments was an augmentation of respiration obtained by injection of a large dose of sodium sulphide after section of the vagi.

Experiment 1 illustrates the respiratory response of a normal animal with its vagi intact to sodium sulphide injection, and furnishes a stand-

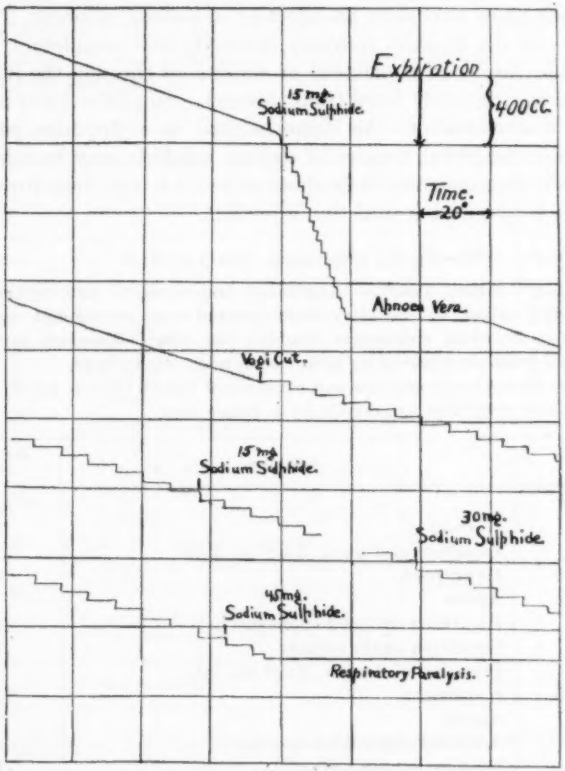


Fig. 1. Showing quantitative records, obtained by the method described, of the respiration of the animal—dog, 5 kilos—recorded in experiment 2.

ard by which the effects obtained in later experiments may be evaluated. Increasing amounts of sulphide were injected intravenously, while the volume of air breathed was recorded quantitatively by means of a spirometer arranged to collect the expired air. This technique for a quantitative record of respiration is far preferable to the chest pneu-

mographs usually employed which, except as regards time, are not quantitative at all. The writing point is connected with the counterpoise weight of the spirometer, and records such as are shown in figure 1 are obtained. The slope of the curve shows the volume of air breathed in unit time.

The data show that after an injection of sodium sulphide, if the animal does not die at once, recovery is speedy and complete. The H_2S liberated in the blood is oxidized or eliminated through the lungs, and the blood is completely freed from this gas. Sulphides have no cumulative action whatever. As demonstrated in a previous paper (2), many times the lethal amount of sodium sulphide may be injected intravenously in a comparatively short period if a brief time for recovery is allowed between each sublethal injection.

Experiment 1. Showing the respiratory effects of Na_2S

Dog, male, 7 kilos. Trachea cannulated under cocain, and connected with two mica disc valves, so that the animal inspired room air through one and expired into a recording spirometer through the other. External jugular vein exposed and sulphide injected by means of hypodermic syringe.

Note the enormous hyperpnea and consequent apnea from a small dose and the immediate respiratory paralysis by a larger dose.

TIME	REMARKS	AIR VOLUME EXPIRED
minutes		*cc. per minute*
1–10	Normal	1300
10	Injection of 2 mgm. Na_2S per kilo	
10–10.5	Hyperpnea	4400
10.5–11	Apnea	0
11–15	Breathing resumed spontaneously	1200
15–20	Condition again normal	1350
20	Injection of 4 mgm. Na_2S per kilo	
20–20.3	Hyperpnea	11600
20.3–21	Apnea	0
21–25	Artificial respiration necessary	
25–30	Respiration normal	1400
30	Injection of 12 mgm. Na_2S per kilo. Followed by a few gasps and paralysis of respiration in inspiratory position	0
34	Heart stopped	

As recorded in experiment 1, the respiration was augmented 5- to 10-fold following the injection of sublethal amounts of sodium sulphide. Each period of respiratory augmentation was followed by a definite

period of apnea vera. After the administration of 4 mgm. of sodium sulphide per kilo (6 mgm. per kilo is the immediately lethal amount) the hyperpnea was so intense and the subsequent apnea so prolonged that it was necessary to resort to artificial respiration; otherwise the apnea would have been fatal. In the third and last injection, twice the lethal dose was given and was followed by a few gasps and then paralysis of respiration. Up to a certain limit sulphide stimulates and beyond that it paralyzes respiration.

Experiment 2. Showing the lack of effect of Na_2S after vagus section.

Dog, male, 5 kilos. Arranged as in experiment 1. After 25 minutes the vagi were cut. Note that after vagus section sulphide does not increase the breathing but large doses paralyze the center.

TIME	REMARKS	AIR VOLUME EXPIRED
minutes		*cc. per minute*
5–10	Normal	600
10	Injection of 3 mgm. Na_2S per kilo	
10–10.5	Violent hyperpnea	3700
15.5–16	Apnea	0
16–20	Breathing resumed spontaneously	450
20–25	Normal	600
25	Vagi blocked with cocain and cut	
25–30	Vagal type of breathing	500
30	Injection of 3 mgm. Na_2S per kilo	
30–35	No perceptible alteration of breathing	500
35	Injection of 6 mgm. Na_2S per kilo	
35–40	No perceptible effect other than some convulsive movements of the legs	550
40	Injection of 9 mgm. Na_2S per kilo	
40.5	Respiration ceased gently	
46	Heart stopped	

In experiment 2 a similar procedure was carried out after the vagi had been cocainized and cut. The response to injection of sodium sulphide was strikingly different from that when the vagi were intact. The repeated injection of sodium sulphide produced no augmentation of the volume of air breathed other than a slight general increase due to the hastened metabolic rate following some convulsive muscular movements. Even the lethal amount of sodium sulphide did not cause a definite respiratory response. Double the lethal amount was, however, followed by death due to cessation of respiration caused apparently by a depressant action upon the respiratory center. When CO_2 is adminis-

tered to a dog after section of the vagi, a 2- or even 3-fold augmentation of breathing is obtained. This fact, originally demonstrated by Scott, was readily confirmed by us and demonstrates that the respiratory center is capable of responding to a proper stimulus even after the vagi have been cut. If, therefore, H_2S had a similar stimulating effect upon the respiratory center, section of the vagi should not completely obscure it.

The conclusion to be drawn, therefore, from experiments 1 and 2, which are chosen from a number of closely similar experiments bearing out the findings in every detail, is obviously that H_2S in the blood up to a certain limit of concentration is a respiratory stimulant chiefly through an action upon the vagal endings in the lungs. In suitable concentrations this action may become so intense that respiration is stopped in the inspiratory position. The action exerted by the gas upon the respiratory center is mainly depressant (in only one experiment was there an exception after vagus section) as is apparent in the effects of high concentrations of sodium sulphide on vagotomized dogs.

The influence of sulphide upon the acid-base equilibrium of the blood. The findings discussed up to this point indicate but one phase in the action of H_2S. A disturbance of the acid-base balance of the blood, even if it does not occur primarily, must result secondarily whenever the respiratory action of the sulphide is exerted.

This is shown by experiments 3, 4 and 5 in which arterial blood samples were drawn before and after the injection of the sulphide. The increased respiration is here seen to be accompanied by a drop in the CO_2 content of the blood. Contrasting sharply is the slight loss in alkali as measured by the change in the CO_2-combining power of the blood measured at 40 mm. With the beginning of apnea the discrepancy between the content and combining power is striking, and indicates a marked decrease in C_H and increase of alkalinity. During the period of apnea, the CO_2 reaccumulates and the content rises toward its normal figure.

Experiment 3. Showing the influence of H_2S upon blood gases and alkali.

Dog, male, 14 kilos. By means of a mask the animal was made to breathe air containing 20 parts of H_2S in each 10,000, i.e., 0.2 per cent. The gas mixture was passed in a continuous stream through the mask. The general behavior was observed. The femoral artery was exposed and cannulated under cocain anesthesia. Frequent blood samples were drawn and determinations made of the CO_2 content of the blood and the CO_2 combining power at 40 mm. The O_2 content of the arterial blood was also determined.

Note the over-breathing, reduction of CO_2, apnea, slight decrease of alkali, anoxemia, death.

TIME	ARTERIAL BLOOD			
	Behavior of animal	CO_2 content	CO_2 combining power at 40 mm.	O_2 content
minutes		*volumes per cent*	*volumes per cent*	*volumes per cent*
0	Normal	54	52	16
3	Inhaling 0.2 per cent H_2S. Violent hyperpnea			
8	Respiration stopped	22	51	16
10	Apnea	36	47	6
15	Apnea	44	47	2
16	Death			

Experiment 4. Showing effect of inhalation of H_2S with recovery.

Dog, male, 11 kilos. General procedure same as in experiment 3. The animal was made to breathe 10 parts of H_2S in 10000 of air.

Note the hyperpnea and lowering of CO_2, and consequent decrease of alkali, followed by recovery.

TIME	REMARKS	ARTERIAL BLOOD		
		CO_2 content	CO_2 combining power at 40 mm.	O_2 content
minutes		*volumes per cent*	*volumes per cent*	*volumes per cent*
0	Normal	56	56	14
	H_2S started			
4	Violent dyspnea			
15	Animal stopped breathing. H_2S off	14	44	14
17	Apnea	28	41	6
18	Animal breathing again			
19	Breathing fresh air only	46	41	13
60	Animal apparently in normal condition	53	52	15

During the period of low CO_2 there is a slight drop in the alkali of the blood. There can be little doubt that this is in the nature of the compensatory change, or acapnial process (4), described previously by us. There are two reasons for this conclusion. *1*, The injection of sodium sulphide adds more alkali than acid. It is an alkaline salt. Even if the liberated H_2S were completely oxidized to form Na_2SO_4 with

the blood alkali, the lethal amount of sulphide could not produce enough sulphuric acid to combine with more than a few milligrams of sodium. On the contrary, following the injection of Na_2S the alkali of the blood is, theoretically at least, increased by the sodium liberated by the hydrolysis of the sulphide. *2*, The decrease of CO_2 and alkali in the blood does not occur in vagotomized animals; in them there is no respiratory augmentation. The conclusion seems obvious that the fall in blood alkali is due, not to neutralization by acid (the acidotic process), but to the passage of alkali out of the blood to compensate an otherwise excessive alkalinity.

Experiment 5. Showing effects of inhalation of H_2S on vagotomized dog.

Dog, male, 12 kilos. The procedure was in every way identical with that of experiment 3 except that the vagi had been exposed under cocain anesthesia, blocked with cocain and then cut.

Note the absence of over-breathing, but on the contrary depression of breathing and asphyxial alteration of blood gases without appreciable change of blood alkali.

TIME	REMARKS	ARTERIAL BLOOD		
		CO_2 content	CO_2 combining power at 40 mm.	O_2 content
minutes		*volumes per cent*	*volumes per cent*	*volumes per cent*
0	Vagi cut	46	50	17
15	Inhaling 0.2 per cent H_2S, no perceptible effect	48	51	16
26	Respiration noticeably decreased			
28	Respiration very weak	57	50	4
30	Respiration stopped	61	49	2
34	Heart stopped			

The course of events may be summarized as follows: The administration of sulphide in sufficient amounts stimulates the respiration through an action upon the vagi, presumably the nerve endings in the lungs. As a result of this augmentation of respiration carbon dioxide is blown off from the blood and the C_H of the blood is lowered. When an alkalosis of sufficient degree has been produced, the stimulating action of the H_2S is counteracted and an apnea follows. Before and during the apnea the H_2S in the blood is oxidized. Meanwhile CO_2 reaccumulates in the blood from the metabolism of the animal. When a sufficient C_H has been thus restored, respiration

occurs spontaneously. This is aided by the acapnial decrease in the alkali of the blood which lowers the level to which the CO_2 must return in order to bring the CO_2 ratio (H_2CO_3:$NaHCO_3$) back to normal. If the loss of CO_2 has been very great the time required for it to reaccumulate is so long that acute anoxemia develops and the animal dies of fatal apnea vera.

The process here described is similar in many respects to that induced by CO poisoning. After CO also the over-breathing is due apparently to stimulation in which the vagi play an essential part and the blood alkali is lowered through the acapnial, not the acidotic, process (4).

CONCLUSION

Sulphides in small amounts in the blood are oxidized,—as shown in a previous paper. In slightly larger amounts (0.1 in 100 of air) hyperpnea is induced. Sodium sulphide (2 mgm. per kilo) when injected intravenously liberates H_2S in the blood and induces hyperpnea followed by apnea vera. This does not occur (with one exception among many experiments) after section of the vagi.

Apparently, therefore, the stimulating action of sulphide upon respiration is chiefly due to irritation of the afferent endings of the pulmonary vagi. Upon the respiratory center small amounts of sulphide are generally without perceptible effect. Larger amounts paralyze respiration.

Hydrogen sulphide is so powerful pharmacologically that the dosage is much too small to affect the C_H of the blood directly. During the hyperpnea which sulphides induce, an excessive loss of CO_2 occurs; and the blood alkali is reduced by the acapnial process just as in the hyperpnea due to ether, low oxygen, carbon monoxid, or nervous excitement.

BIBLIOGRAPHY

(1) Loewenhart, Malone and Martin: Journ. Pharm. Exper. Therap., 1922, xix, 13.
(2) Haggard: Journ. Biol. Chem., 1921, xlix, 519.
(3) Scott: Journ. Physiol., 1908, xxxvii, 301.
(4) Haggard, and Henderson: Journ. Biol. Chem., 1921, xlvii, 421.

THE EFFECTS OF PROSTATE SUBSTANCE ON THE METAMORPHOSIS OF THE INTESTINE OF FROG TADPOLES

ROBERT W. HEGNER

From the Department of Medical Zoölogy, School of Hygiene and Public Health, Johns Hopkins University

Received for publication May 6, 1922

Macht (1), (2) reports that the feeding of desiccated and powdered prostate gland substance from the ram, bull, steer and man, stimulates both growth and metamorphosis of the larvae of frogs, toads and salamanders. The species used were *Rana sylvatica*, *R. palustris*, *R. catesbeiana*, *Bufo lentiginosus* and *Ambystoma punctata*. The tadpoles experimented on were either just hatched or from one day to three weeks old. These feeding experiments, he states, are the first to show a relation of prostate feeding to the growth of animals and indicate that the prostate gland produces an internal secretion.

During some feeding experiments carried on by the writer to test the effects of diet on intestinal protozoa, desiccated, powdered substance from the prostate of the ram prepared by Armour & Company, was fed to tadpoles. These consisted of two-year-old tadpoles of the bull frog, *Rana catesbeiana*, and of one-year-old tadpoles of the green frog, *Rana clamata*. A method of feeding similar to that used by Swingle (3) was employed. The tadpoles were divided into two lots. The controls were fed on flour that had been made into paste by the addition of water, spread thinly on glass plates to dry, and then crumbled. The experimental tadpoles were fed on 7 parts flour plus 3 parts desiccated prostate substance, prepared in a similar way. The tadpoles were kept in glass stender dishes 9 inches in diameter and $3\frac{1}{4}$ inches deep, twenty in each dish, and provided every day with one liter of fresh tap water. Feeding began on April 11, 1922, the day the tadpoles were collected. The experimental animals exhibited no ill effects from their food. Externally the two sets of animals did not exhibit conspicuous differences 17 days after the experiment was begun, although those fed on prostate substance had hind legs that were slightly better developed and tails that seemed slightly shorter than those of the controls. Studies of the

effects of thyroid feeding on the metamorphosis of tadpoles have shown that one of the most noticeable results of this treatment is the shortening and differentiation of the alimentary canal from the stomach to the posterior end. It was decided, therefore, to compare the alimentary canals of the two sets of tadpoles. On April 28 ten controls and ten experimental animals were selected in pairs so that the size and age of the control would be as nearly as possible like that of the experimental tadpole with which it was contrasted, and on May 1, two more sets of ten each were selected in a similar way. The measurements of the intestines of these twenty pairs of tadpoles from the posterior end of the stomach to the anal aperture are as follows:

TABLE 1

Length of intestine of paired specimens of control and prostate-fed tadpoles, in centimeters. Specimens 1 to 4 were of R. catesbeiana and 5 to 20 of R. clamata

NUMBER	CONTROL	PROSTATE	NUMBER	CONTROL	PROSTATE	NUMBER	CONTROL	PROSTATE	NUMBER	CONTROL	PROSTATE
1	62	57	6	22	10	11	33	31	16	28	15
2	64	46	7	34	18	12	30	18	17	32	16
3	60	56	8	31	14	13	33	20	18	30	19
4	60	52	9	39	31	14	36	19	19	24	11
5	36	25	10	34	21	15	32	18	20	26	17
Totals										746	514

In every case the intestine of the experimentally-fed tadpole was shorter than that of the control, and in many cases it was only about one-half as long. The total length of the intestines of the controls was 746 cm., that of the prostate-fed tadpoles, 514 cm. The average length was 37.3 cm. for the controls and 25.7 cm. for the prostate-fed specimens. The shortening of the intestine in the bull frog tadpoles (the first four in the table) is noticeably less than in the green frog tadpoles, the averages here being 61.5 cm. for the controls and 53 cm. for those fed on prostate substance. This result may be due to the greater age of the bull frog tadpoles.

These results confirm those obtained by Macht (1), (2) on very young tadpoles and indicate that the prostate material contains something that stimulates the differentiation of the intestine of bull frog and green frog tadpoles.

BIBLIOGRAPHY

(1) Macht: Proc. Soc. Exper. Biol. and Med., 1919, xvi, 138.
(2) Macht: Journ. Urology, 1920, iv, 115.
(3) Swingle: Journ. Exper. Zoöl., 1919, xxiv, 521.

STUDIES UPON THE MECHANISM OF THE INCREASED METABOLISM IN HYPERTHYROIDISM[1]

JOSEPH C. AUB, ELIZABETH M. BRIGHT AND JOSEPH URIDIL

From the Laboratories of Physiology of the Harvard Medical School

Received for publication May 9, 1922

Good evidence of the mechanism by which the active principle of the thyroid gland exerts its influence on metabolism is still scanty. Magnus Levy (1) accounts for the increased metabolism in Basedow's disease by increased heart and respiratory activity, and finds that the resting metabolism is increased. Speck (as quoted by Magnus Levy) thought that the trembling largely accounted for the increase, and that this explanation is possible has been shown by the recent work of Langley (2), who found that denervation of muscles with a resulting fibrillation causes a very large increase above the resting metabolism. Marie Krogh (3) studied the metabolism in urethanized frogs after the administration of thyroidin. Her methods were somewhat similar to those we have employed. She found that the metabolism rose even though the animals were anesthetized throughout the experiments, but thought that this rise did not appear after the nerves to the muscles had been cut. She therefore concluded that in these preliminary experiments the increased metabolism was due to muscle tonus. There is also evidence, suggested by Herring (4), Levy (5), Cannon and Cattell (6) and others that there is distinct relation between the thyroid and adrenal glands, though no relationship has yet been established in regard to their metabolic activities (7).

The present experiments were undertaken to study further the mechanism of the increased heat production after thyroid ingestion, to see whether muscular movements, the fibrillation of muscles, muscle tonus, or adrenal secretion could be its cause, or whether it was necessary to assume that the general basal rate of cellular combustion was raised.

[1] This work was first announced at the symposium on Basal Metabolism at the meeting of the American Medical Association, Boston, June, 1921.

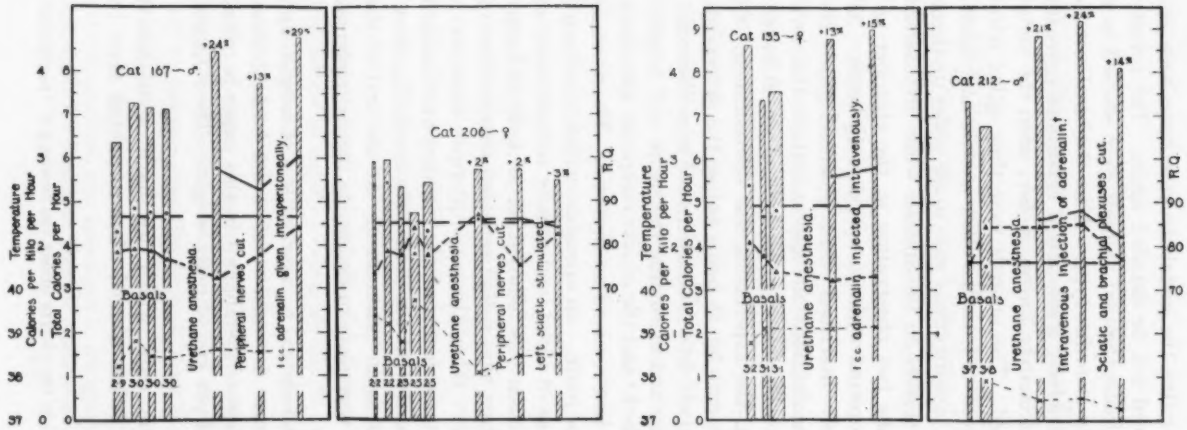
The methods used to determine the metabolic rate have been described elsewhere (8), and need not be detailed again. The investigation was made on cats. Unanesthetized animals were studied in a closed-system apparatus of the Benedict type. After the basal metabolism had been carefully determined, a solution of crystalline thyroxin was injected in doses of approximately 3 mgm. a day, until the metabolic rate was markedly raised. The animals were then either adrenalectomized and allowed to recover from the ether anesthesia (expers. 165, 201 and 204) or the final investigations were made under urethane anesthesia. The respiratory studies on the animals while urethanized followed immediately after the last observation in the chamber apparatus, and were always completed within a period of 4½ hours. All of the precautions previously described were observed (8). The temperature was carefully maintained at approximately the level found before anesthetization. The blood pressure level was followed, and if it fell to 80 mm. Hg. the experiment was discontinued. Of course, no food was given the cats for 18 hours before the determinations were made, and periods in which the animals moved were discarded.

The effects of adrenalectomy and of cutting the sacral and brachial nerve plexuses were then studied,[2] and the results compared with those obtained from animals which had received no thyroxin. The adrenals were removed by the posterior route,—an operation which is attended by practically no shock, no loss of blood, and usually without opening the peritoneum. The metabolism was followed for an hour or longer after this procedure, and during this period repeated observations of the metabolic rate were made. When the nerve plexuses were cut, a small incision was made near the spinal cord, the plexuses were isolated and ligatured in two places and the cut was made between the ligatures. Then the metabolic rate was again determined. At the end of the experiment a careful autopsy was made to determine the completeness of the operations.

The injection of thyroxin is followed by marked loss of weight, and by rise of the total metabolism. In order to allow for both of these factors, the percentage variation of metabolism in this paper is calculated in terms of calories used per kilo of body weight. These figures may be found on the charts.

General considerations. The data which we have accumulated involve so many figures that it has seemed wiser to publish them in charts rather than in tables.

[2] This procedure is abbreviated in the charts under the heading of "peripheral nerves cut."



Group I. The effects of anesthesia, adrenalectomy and denervation of muscles upon the metabolism of the normal cat.

The cross hatched rectangles represent the total calories per hour, their width indicating the number of quiet periods which were included in the average figures. The first observation on cat 206 represents one period.

The circular points represent calories per kilo.

The connecting solid black lines represent (1) the average normal calories per kilo,—the horizontal line—and (2) the calories per kilo used during the variations induced by the experiment.

The triangular points and heavy broken connecting lines represent the respiratory quotients.

The crosses and light broken lines represent the temperature of the animal at the time of observation.

The percentage variation of metabolism given at the top of the cross hatched rectangles refers to variations per kilo of body weight.

The figures at the base of the cross hatched rectangles are the weight of the animal at the time of observation.

The effect of anesthesia on the metabolic rate is not well known, and in these experiments it was important to know what its normal effect would be. Tangl (9) had studied one rabbit before and after urethane anesthesia and obtained a slight drop in the metabolism after anesthesia. The four control experiments on our normal cats show that urethane anesthesia causes a rise of 15 per cent above the normal basal metabolism, a fact which may be explained by the activity of the adrenal glands. This is discussed in the next paper. A similar rise occurs in most of the thyrotoxic animals so that the average change in the metabolism in the ten experiments here recorded was 13.2 per cent increase after urethane anesthesia. Our general experience in animals which were only studied in either the anesthetized or unanesthetized state gives the general average of figures for the meta-

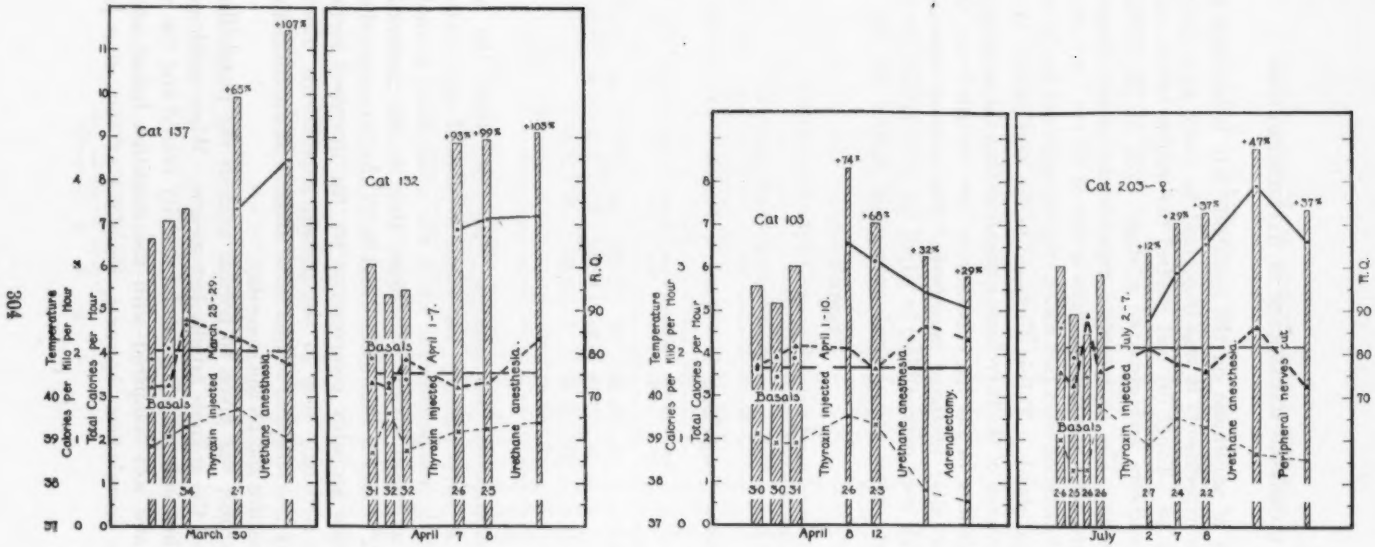
TABLE 1

Normal basal metabolism under different conditions

TYPE OF ANESTHESIA	AVERAGE CALORIES PER KILO PER HOUR								AVERAGE TEMPERATURE
	Total experiments		Male cats		Female cats		Sex not noted		
	Number	Calories	Number	Calories	Number	Calories	Number	Calories	
Unanesthetized.....	54	2.28	17	2.28	19	2.27	18	2.28	39.2
Urethane.....	54	2.20	32	2.18	13	2.20	9	2.34	37.2
Decerebrate.....	13	2.41	5	2.39	8	2.43			37.6
Paraldehyde.....	2	2.41							37.3

bolic rate summarized in table 1. In this series the average metabolism is approximately the same in normal and urethanized animals, but the temperature at which the urethanized animals were maintained averaged about 2°C. lower than in the unanesthetized animals (37.2°C. for the anesthetized and 39.2°C. for the unanesthetized). This difference probably compensates for the increased metabolism which is so strikingly seen in the present experiments where both methods may be compared on the same animals at approximately the same temperature and on the same day.

When thyroxin was given to normal animals the metabolism rose markedly and the animals rapidly lost weight. When urethane anesthesia was then induced, all muscular activity ceased and the muscles felt flabby and soft compared with the condition before anesthetization. The metabolism, however, remained at the same level or



Group II. The effects of anesthesia, adrenalectomy and denervation of muscles upon the metabolism of the thyrotoxic cat.

rose even higher than before anesthetization, and the conclusion seems justified, therefore, that neither muscular movements nor marked tonus can be the explanation of the increased combustion following ingestion of thyroxin.

Changes in the pulse rate are shown in table 2. They are not of sufficient magnitude to explain so marked a rise of metabolism.

A graphic index of muscular tremors was sought. Electromyograms both with and without the audion bulb[3] were made through the intact shaved skin and involuntary muscular movements were studied

TABLE 2

The effect of thyroxin upon the heart rate

NUMBER OF ANIMAL	DATE	WEIGHT	PULSE		
			Basal	After thyroxin	After urethane
	1921				
154	April 30	4.00	140		
	May 5	3.60		156	180
162	April 13	2.50	136		
	April 30	2.10		260	214
132	April 1	3.24	160 ±		
	April 8	2.54		254	252 – 240
137	March 23	3.43	154		
	March 30	2.71		266	240 – 252
105	March 31	3.1	130		
	April 8	2.6		274	
	April 12	2.28			

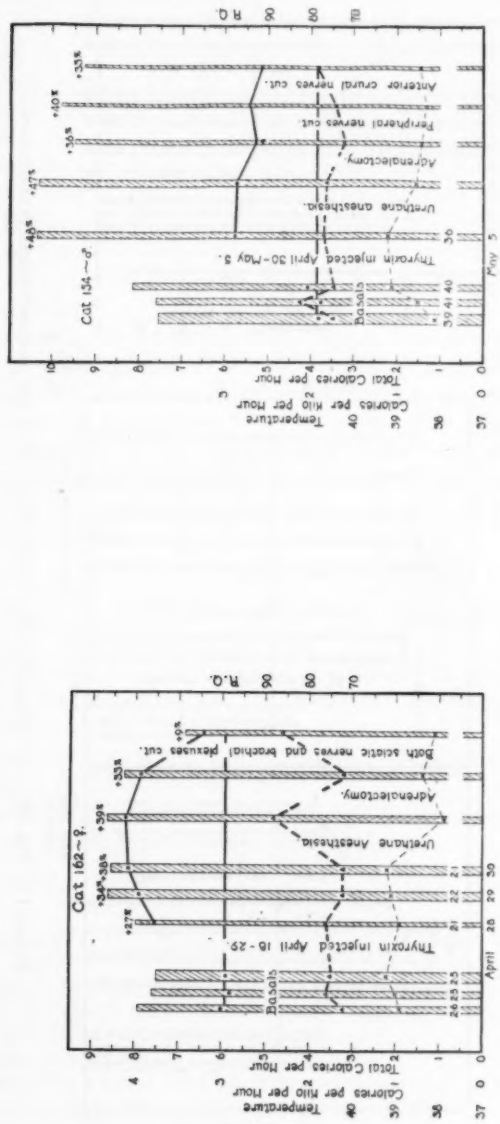
in the relaxed animal before and after both thyroxin and anesthesia. No consistent results were obtained which would justify the conclusion that, in the cat, thyroxin causes any marked twitching or fibrillation of the muscles.

In order to be certain that tonus is not the cause of the elevated metabolic rate and may therefore be wholly eliminated as a factor, we next cut the chief plexuses going to the muscles, i.e., the sciatic and brachial plexuses. Impulses from both the central and sympathetic

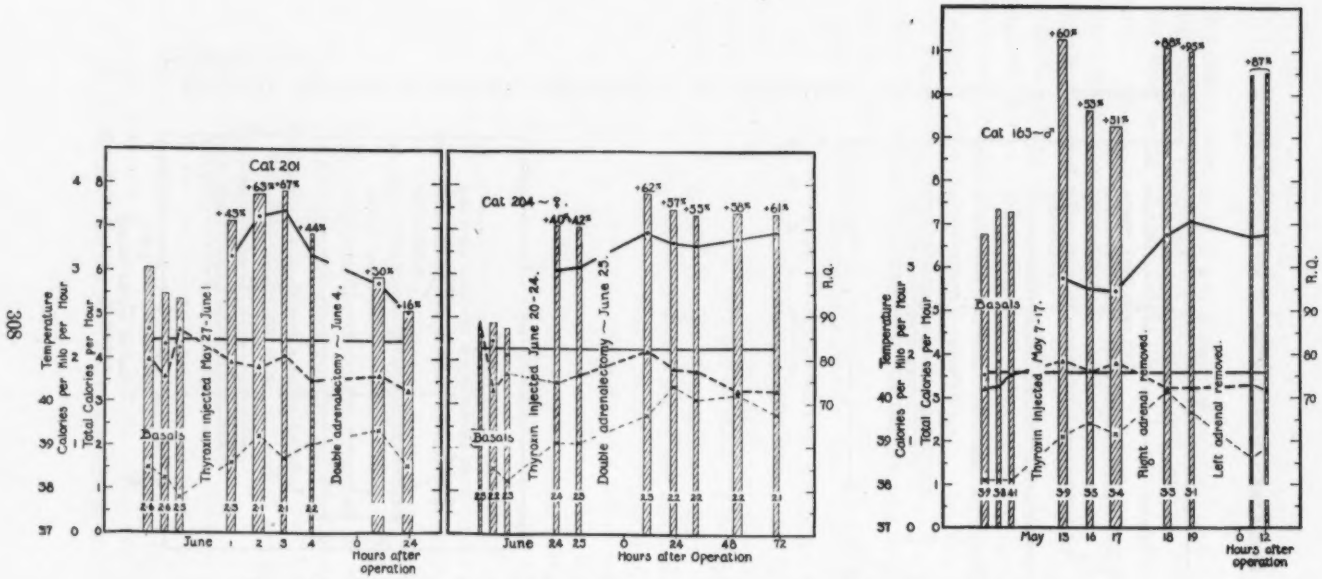
[3] These observations were made possible by the kind coöperation of Dr. Alexander Forbes.

nervous systems were in this way largely eliminated and most of the limb muscles were paralyzed. This was done because there is still some question as to whether slight tonus is not maintained under anesthesia. Mansfeld and Lukacs (10) claim that tonus may be maintained by sympathetic stimuli, for if the nerves to the hind leg are cut in a curarized animal the metabolism falls, but if the sympathetic ganglia are first cut no such metabolic decrease follows sectioning the peripheral nerves. They therefore conclude that some tonus may be maintained in curarized animals, and that the elimination of this tonus causes a reduction of the metabolic rate. But this work has not been confirmed. Recent work on the effect of the sympathetic nerves upon the oxygen consumption of the leg muscles of the cat has been done by Nakumura (11), who cut the sciatic and lumbar sympathetic nerves in urethanized and decerebrate cats. Such section did not decrease the consumption of oxygen, and he concludes, therefore, that if the sympathetic system has any tonic action on muscle, this tone does not increase the amount of oxygen used. Similar conclusions were reached by Frank and Voit (12) and by Roaf (13) who eliminated extensor tone by means of curare. By stimulation of the sympathetic cords Nakumura obtained a marked slowing of the blood flow and a decrease in the amount of oxygen used. In the thyrotoxic frog, Marie Krogh after severing the peripheral nerves found a fall in metabolism and therefore concluded that tonus might be the explanation of the increased metabolism. In metabolism determinations, in mammals at least, the blood pressure must not fall below 80 mm. Hg. If it falls below this level the experiment must be discarded (8). In our control animals we find a distinct drop in the metabolism after section of the nerves, in one animal a drop which is quite analogous to the fall sometimes seen in thyroxinized animals. In thyroxinized animals cutting the nerves sometimes causes a distinctly diminished metabolism, but the maximum drop does not bring the level to the basal values found before thyroxin was given. In cat 154 the cutting of the nerves caused no drop in the rate of metabolism, and this experiment establishes the fact that thyroxin does not affect the metabolic rate by affecting the tonus of the muscles.

That the adrenal glands markedly influence the metabolic rate is shown and discussed in the two following papers. It therefore becomes of interest to know whether the metabolic effects of the thyroid are dependent on the presence of the adrenal glands. This problem was attacked by two methods in thyroxinized animals, first in short experi-



Group III. The effects of anesthesia, adrenalectomy and denervation of muscles upon the metabolism of the thyrotoxic cat.



Group IV. The effect of adrenalectomy (under ether) upon the unanesthetized thyrotoxic cat.

ments on anesthetized animals (expers. 162, 105 and 154) with the technic described in the third paper of this series; and second, by removing the adrenals under ether anesthesia and then following the metabolism (expers. 165, 201 and 204) in unanesthetized animals with the technic described in the second paper of this series.

In both types of experiments the results show that in spite of the absence of the adrenal glands, the increased metabolism due to thyroxin was maintained. It seems clear, therefore, that the metabolic increase caused by thyroxin is not accomplished by means of the adrenal glands, and this is further evidence that the metabolic effects of the two glands are not due to a single mechanism or, at least, that they may work independently.

It has therefore been satisfactorily proven that the increased metabolism due to the administration of thyroxin is maintained in spite of the elimination of all muscular activity of the limbs and in spite of the removal of tonus from these muscles. The adrenal glands are also not essential to the maintenance of the high metabolism. We are therefore again confronted with the theory that the thyroid secretion acts as a draught, stimulating the resting body cells to an increased rate of combustion.

CONCLUSIONS

Urethane anesthesia causes a rise in the metabolism of thyrotoxic animals as it does in normal animals.

The increased basal metabolism stimulated by thyroxin cannot be explained by muscular activity, muscular fibrillation or increased muscle tonus.

The adrenal glands are not essential to the maintenance of a high metabolic rate induced by thyroxin.

These findings support the theory that thyroxin stimulates resting cells directly to a higher level of combustion.

BIBLIOGRAPHY

(1) Magnus Levy: von Noorden's Handb. d. Stoffwechsels, Zweite Auflage., ii, 1907.
(2) Langley and Kato: Journ. Physiol., 1915, xlix. 432.
Langley and Itagaki: Journ. Physiol., 1917, li, 202.
(3) Krogh: Ugesk. f. Laeger, 1916, lxxviii, 2337.
(4) Herring: Endocrinol., 1920, iv, 577.
(5) Levy: This Journal, 1916, xli, 492.
(6) Cannon and Cattell: This Journal, 1916, xli, 74.

(7) Boothby and Sandiford: This Journal, 1920, li, 200.
(8) Aub: This Journal, 1920, liv, 388.
Aub, Forman and Bright: This Journal, 1922, lxi.
(9) Tangl: Biochem. Zeitschr., 1918, xcii, 318.
(10) Mansfeld and Lukacs: Arch. f. d. gesammt. Physiol., 1915, clxi, 467.
(11) Nakumura: Journ. Physiol., 1921, lv, 100.
(12) Frank and Voit: Zeitschr. f. Biol., 1901, xlii, 309.
(13) Roaf: Quart. Journ. Exper. Physiol., 1912, v, 31.

THE RELATIVE GROWTH OF INDIVIDUAL BOSTON SCHOOL BOYS[1]

W. T. PORTER

From the Laboratory of Comparative Physiology in the Harvard Medical School

Received for publication May 8, 1922

I

No one will deny that the growth of children may be greatly improved by intelligent care. To this end, standards are necessary. The physician, or other adviser, must be sure of the normal rate of growth in order to recognize and assist the child whose growth is beginning to fail. But the normal rate of growth is hardly known at present. It is true that the average size of children at each age is accurately placed, but this will not serve. The rate of growth in weight from age eight to age nine, for example, cannot be had by subtracting the average weight at age eight from that at age nine. The difference thus obtained is one year's increase in an average; it is a statistical and not a personal growth. This vital distinction is clearly illustrated by table 1.

In table 1 are the weights of nine boys at ages 8 and 9. They average 55 and 61 pounds, respectively, which is the average weight of all Boston

[1] The author's earlier studies in the growth of children are: *The physical basis of precocity and dullness.* Transactions of the Academy of Science of St. Louis, 1893, vi, 161–181, *Untersuchungen der Schulkinder in Bezug auf die physischen Grundlagen ihrer geistigen Entwickelung.* Verhandlungen der Berliner Anthropologischen Gesellschaft, 15 Juli, 1893. Zeitschrift für Ethnologie, ss. 337–354. *The relation between the growth of children and their deviation from the physical type of their sex and age.* Transactions of the Academy of Science of St. Louis, 1893, vi, 233–250. *On the application to individual school children of the mean values derived from anthropological measurements by the generalizing method.* Quarterly publications of the American Statistical Association, December, 1893. *The growth of St. Louis Children.* The Transactions of the Academy of Science of St. Louis, 1894, vi, 263–380, 46 plates. *The seasonal variation in the growth of Boston school children.* This Journal, 1920, lii, 121–131.

A substantial part of the cost of the study on seasonal variation as well as of the present study has been paid by the Permanent Charity Fund, whose assistance is gratefully acknowledged.

school boys at ages 8 and 9. Examine the weights of these boys. At age 8, William is heavier than 90 per cent of other boys, and Charles is heavier than 10 per cent; their mates are ranged between. Turn now to age 9. Within a year, much may happen. Some of these boys have been fortunate in growth and some unfortunate. Richard, who was heavier than 70 per cent, has lost his place to George, who at age 8 was heavier than only 30 per cent. Stephen, who happened to be at the average when aged 8, has been replaced at age 9 by Alfred. Obviously, in such a table, the weights and the names temporarily attached to them are of two different categories, statistically unrelated. In the case before us, the growth of the average from 55 to 60 pounds is not the growth of any individual.

TABLE 1

NAME OF BOY	WEIGHT AT AGE 8	PERCENTILE GRADE	WEIGHT AT AGE 9	NAME OF BOY
	lbs.	*per cent*	*lbs.*	
William	64	90	71	Henry
Henry	61	80	67	William
Richard	58	70	64	George
John	57	60	62	James
Stephen	55	50	60	Alfred
James	53	40	59	John
George	52	30	57	Richard
Alfred	50	20	55	Stephen
Charles	47	10	52	Charles
Average.....	55		61	

In table 1, the weights are those determined by the present investigation, but the names are chosen arbitrarily. Yet table 1 faithfully imitates cases actually in our records. Nine of these cases are given in figure 1. To the left, in this figure, are two pairs of curves: the lower pair shows three years' growth in the height of Alma F. and Anna Y.; and the upper pair three years' growth in the height of Edward P. and Walter J. The average in each of these pairs would be a straight line, and the increase of this average from year to year would give a wrong idea of the growth of any one of these children.

In the middle of figure 1 is the history of Rosa Martin and Dorothea White. Rosa, at age 7, was heavier than 35 per cent of girls of her age, and Dorothea was heavier than 68 per cent. At age 13, they had exchanged places. These personal vicissitudes do not affect the average;

the name of the girl in the 35 percentile grade at age 7 or at age 13 is of no consequence to the statistical mean.

To the right in figure 1 are percentile grades of three boys from age 6 to age 10. Thomas Reddington, in the center, remains through the four years in the same percentile grade; at age 6, he was heavier than 95 per cent of boys of his age, and at ages 7, 8, 9 and 10, he still was heavier than 95 per cent. John McCarthy, at age 6, was heavier than 83 per cent of other boys, and Samuel Neuser was heavier than only 4 per cent—a very small boy indeed. The curves of all three have been

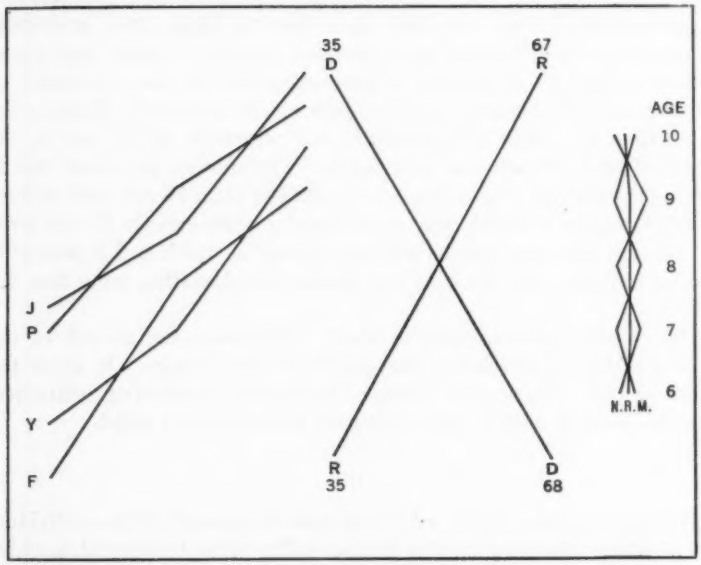


Fig. 1

brought close together for convenience of comparison. M. loses ground at ages 7 and 9, and gains ground at ages 8 and 10; while N. gains at ages 7 and 9, and loses at ages 8 and 10. Who shall say, from an average, what should be the normal growth of any one of these three boys? Nor would the median be more helpful than the average. The fallacies of the average apply equally to any other central value.

Consider the task of the physician. Parents ask: "Are my boys John, Samuel and Thomas growing as fast as they should, or must their growth be stimulated?" The physician, physical director, or

other counselor must answer this question. Figure 1 demonstrates that he cannot rely alone upon the standard curves now in use, because these curves express the average at each year, and the increase of the average from year to year is not necessarily the normal annual growth of the child who seeks counsel. In figure 1 an average or standard rate of growth would not be the rate of growth of any of the nine children, except Thomas Reddington, who remains at the average for the 95 percentile grade.

To remove this fundamental difficulty, urged by me in 1893, the present investigation was begun in 1910. It deals with the personal histories of 2421 boys and 2380 girls, born in 1904, 1905, and 1906, whose weights and heights were recorded monthly[2] during nine years. This investigation establishes a practical basis for the treatment of defective growth because it determines *a*, the numerical chance—the odds—that any child will remain in his percentile grade: and *b*, the degree of such deviation as may occur. The chances are about one in seven, for example, that a boy who is heavier than 75 per cent of boys at age 8 will be at age 9 once more heavier than exactly 75 per cent; the chances are even that he will not deviate as much as 2.5 percentile grades; and they are three to one against his deviating more than 4.5 grades.

The present investigation, in short, determines the growth of the individual boy in relation to the growth of the average. It gives the limits within which the former standards—expressing statistical growth—may be used in prescribing for the individual child.

II

The method here employed to discover the growth of the individual boy relative to the growth of the average is illustrated by figure 2, in which William Hoerrner's growth in height is plotted on a background of percentile grades. At age 6, this boy was taller than 82 per cent of boys of his age. During the next five years he grew somewhat faster than the comrades who, like himself, had been at age 6 in the 82nd percentile grade. Thus at ages 7, 8, 9, 10 and 11, William H. was found in grades 85, 87, 89, 90 and 91, respectively. At age 12, he had fallen to grade 87; and at age 13, he was back in 82, his grade at age 6. The deviations of William H. from year to year were 3, 2, 2, 1, 1, 4, 5 grades. Obviously, when the personal deviations of all the boys are properly grouped, the chance

[2] Subject, of course, to absences at the time of measurement.

that any boy will deviate more than a certain number of grades can be accurately calculated. Similarly, it is possible to calculate the degree of deviation from ages 6 to 7, 6 to 8, and so on to ages 6 to 13.

Concerning the background of percentile grades, special students in this field will pardon a word or two which may refresh the memory of readers who have not lately pursued such calculations. The per-

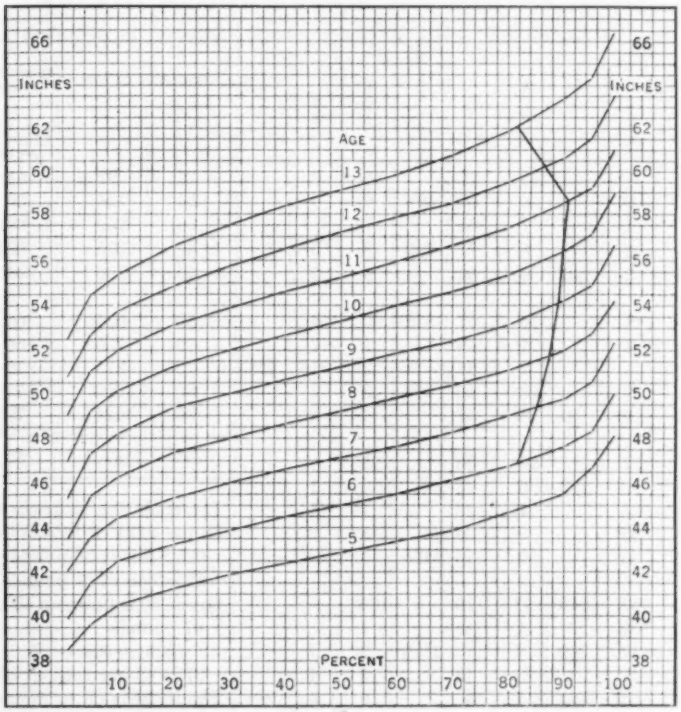


Fig. 2

centile grades in this investigation were figured for each month of age. In table 2, for example, are given the percentile grades in the weights of 1250 boys aged 125 months. At the left of the table are set down the number of boys who are 45, 46, 47, etc., pounds in weight; to the right are the per cents of the total number of boys—5, 10, 20, etc. per cent of 1250. Let us now calculate the twenty percentile grade. Twenty

per cent of 1250 is 250. On adding the boys at each pound, beginning with the smallest, we reach 240 at pound 60. Now 240 is less than 250; the twenty percentile grade is therefore more than 60 pounds. But, if the next group of boys were added, the total would be 292, which is more than 20 per cent; hence the twenty percentile grade is not so

TABLE 2

The percentile grades in the weights of 1250 boys aged 125 months

POUNDS	BOYS	NEAREST SUM	POUNDS	BOYS	NEAREST SUM	POUNDS	BOYS	NEAREST SUM	PER CENTS OF 1250		PERCENTILE GRADES
											per cent
45			65	71		85	6		5	62.5	54.2
6	2		6	65	598	6	6		10	125	56.6
7	1		7	60		7			20	250	60.2
8	2		8	71	729	8	3		30	375	62.5
9	5	10	9	56		9	5		40	500	64.5
50	11		70	57	842	90	4		50	625	66.5
1	6		1	49		1	7		60	750	68.4
2	11		2	35		2	1	1236	70	875	70.7
3	9		3	40	966	3	5		80	1000	73.9
4	9	56	4	37		4	2		90	1125	79.0
55	27		75	30		95			95	1187.5	82.2
6	29	112	6	25		6	1				
7	23		7	28		7	1				
8	29		8	26		8	2				
9	33		9	13	1125	9					
60	43	240	80	29		100					
1	52		1	16		1					
2	57	349	2	15	1189	2	1				
3	48		3	15		3	1				
4	65	462	4	4		4	1				

Interpolation at grade 50
50 per cent of 1250 = 625
625>598 at 66 pounds
625<658 at 67 pounds
Grade 50 is between 66 and 67 pounds
625 − 598 = 27
27 is 45 per cent of 60
Grade 50 is 66.45 pounds

much as 61 pounds. Evidently the desired grade lies on the way between 60 and 61 pounds, somewhere in the group of 52 boys who begin at 60 and end at 61 pounds Arrange these 52 in the order of their weight. As 20 per cent of 1250 is 250 and we had come to the 240th boy when we arrived at 60 pounds, we must go 10 boys farther to reach the twenty per centile grade; in other words, 10 out of 52 or

one-fifth the way from 60 to 61 pounds. So the twenty percentile grade is 60.2 pounds. The remaining percentile grades of month 125 are reckoned in like manner, as are the grades for the other months in age 10. Then the twelve values for each of the grades 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95 and 99 are added together and the totals divided by twelve to get the averages for the year. When all the years

TABLE 3

The deviations of individual Boston school boys from their percentile grades in weight and height

AT YEARS OF AGE FROM	WEIGHT			HEIGHT		
	30 per cent	50 per cent	75 per cent	30 per cent	50 per cent	75 per cent
	Do not deviate more than			Do not deviate more than		
	grades	*grades*	*grades*	*grades*	*grades*	*grades*
5 to 6	0.9	2.8	4.6	0.6	2.2	5.0
6 to 7	1.2	2.6	5.6	1.4	2.8	5.4
7 to 8	0.9	2.3	5.2	1.1	2.1	4.5
8 to 9	0.9	2.3	4.6	0.8	1.8	4.6
9 to 10	0.9	2.3	4.8	0.6	1.9	4.4
10 to 11	1.2	3.0	5.6	0.8	1.9	4.4
11 to 12	1.0	2.4	5.4	0.9	2.1	5.2
12 to 13	1.3	3.7	7.0	1.4	3.8	7.5
Averages	1.0	2.7	5.3	1.0	2.3	5.1
6 to 8	1.4	3.3	6.9	2.0	3.8	8.2
6 to 9	1.9	4.5	9.3	2.6	4.9	8.0
6 to 10	3.0	5.6	10.8	3.0	5.4	8.9
6 to 11	3.5	6.5	11.2	3.4	5.8	9.8
6 to 12	4.1	7.8	12.9	3.6	6.3	14.0
6 to 13				3.0	5.6	13.7
Averages	2.8	5.5	10.2	2.9	5.3	10.1

are done, the results are plotted in a coördinate system in which the ordinates are pounds or inches and the abscissae are percentile grades. The final result is the chart shown in figure 2.[3]

III

The histories of individual boys, each of whom has his deviations recorded on a chart like William Hoerrner's (fig. 2) provide the data

[3] All the curves in this investigation are exactly as they came from the statistical mill—none has been "smoothed" or tampered with in any way.

for table 3, in which are placed the fruits of this portion of our studies. On examining table 3 it will be found that 30 per cent of the boys deviate not more than one percentile grade from year to year.[4] We shall not go far wrong if we say that approximately one-third of the boys do not leave their percentile rank in any one year. In other words, if in any school there are 21 boys aged ten who are bigger than 80 per cent of other boys aged ten, 7 of these boys will still be at age eleven bigger than 80 per cent of other boys, while the other 14 will have either diminished their advantage or increased it. There are in short three chances in nine or, more accurately, three chances in ten that any boy found in a given grade in any year from age 5 to age 12 will be in that same grade a year later. So much for those who do not vary in any one year.

Consider now the two-thirds who do vary. It appears from table 3 that the chances are even that no boy will vary more than 2.7 grades and the chances are three to one that no boy will vary as much as 5.3 grades. The value of five grades, as is readily seen on examining figure 2, depends on the age and size of the boy; at age 10, for example, five grades in the height of an ordinary small boy will be not quite one-half inch, and for an ordinary large boy five grades will be a little more than one-half inch, while for weight the deviations will be about 1.5 and 2.2 pounds, respectively. Compare these figures with the annual growth. Between ages 10 and 11, a small boy will grow 5 pounds in weight and $1\frac{3}{4}$ inches in height, and a large boy will grow 7.5 pounds in weight and 2 inches in height. It is evident that the normal deviations month by month (one-twelfth the annual deviation given in table 3) are within the unavoidable errors of observation. It follows that for monthly measurements the existing standard tables must serve, for the present at least, while for longer periods they should be used only within the limits set by table 3.

Monthly or annual deviations have thus far been discussed. We pass now to deviations during two, three, or more years. They are recorded in the lower part of table 3. The chances are three to one that no boy will have varied as much as thirteen grades at the end of the six years from age 6 to age 12. From this an important deduction should be drawn, namely that small boys tend to remain small, unless they are rescued by a wise adviser.

Finally, table 3 demonstrates that the deviations in height are closely similar to those in weight. Indeed, the correspondence is extraordinary.

[4] One grade in weight exceeds a pound only below grade 5 and above grade 90. One grade in height does not exceed one-eighth inch from grade 10 to grade 90.

IV

It will be useful now to see of what help these truths may be in finding and curing defective children.

We draw at once a sharp line between defects in weight and those in height.

A failure to make the normal gain in weight is an invitation to disease or the evidence of disease already present Tuberculosis is a common disease preceded and accompanied by loss of weight, and there are numerous other examples. Indeed, a failure to make the normal gain in weight is in itself a pathological state. The early recognition of diseased states is an imperative reason for weighing children at frequent intervals, certainly not less often than once a month Should the child fall below his percentile rank, as shown by a chart of percentile grades similar to figure 2, or should the child's pounds per inch be less than those shown in a standard table of pounds per inch, the cause of the defection should at once be sought. A former publication[5] in this series shows that seasonal variations must be taken into account in determining monthly growth in weight. The present investigation brings the comforting assurance that in determining the monthly growth in weight the personal deviation from the growth of the average is small enough to fall within unavoidable errors of observation For determining monthly growth in weight, therefore, the tables already in use need to be corrected only for seasonal variation; monthly measurements do not need to be corrected for personal deviation.

The growth in height should also be taken each month, but the errors of observation are such that a failure to make the normal gain will hardly be evident under two or perhaps three months. There need be no correction for seasonal growth, since seasonal growth in height has not been demonstrated. The personal deviation must however be taken into account for observations including three months or more.

A word should now be said as to the value of height in itself. At the present time, those who speak *ex cathedra* proclaim the supreme importance of weight in relation to height. Indeed, their disciples make a talisman of pounds per inch. The emphasis is overdone, in that it tends to lessen the attention paid to growth in height. A world in which every human being had the correct number of pounds for each inch in height would be the best possible world for adults, in whom

[5] W. T. Porter. The seasonal variation in the growth of Boston School Children. This Journal, 1920, lii, 121.

skeletal growth is hopelessly finished, but it would be a very poor world for public school children, the majority of whom have been deprived of their rightful number of inches. It is our duty to make our children reasonably tall. Again and again it has been proved that life is more difficult for undersized men and women than for those who are somewhat taller than the average. The parental and social incompetence which produces stunted children does not repair its fault by making the pounds of a defective boy bear the customary ratio to his defective

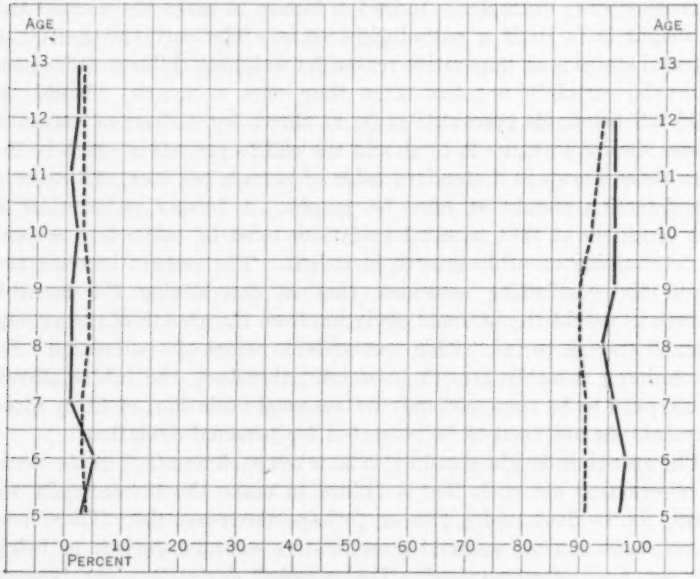


Fig. 3

inches. I repeat that aside from its use in the recognition of approaching or present illness, the gain in weight is not our chief care. Except in sick children, if we get the inches, the pounds will look after themselves.

V

The statement that if we have the inches we shall also usually have the pounds requires examination. It declares a close correlation between height and weight. Figure 3 illustrates this correlation. At

the left in the figure is the history of J. C., a short boy; and at the right is the history of A. T., a tall boy. The unbroken lines mark the percentile heights at each age; the broken lines, the percentile weights. J. C. is at age 7 shorter than 99 per cent of boys of his age and he is lighter than 97 per cent; A. T. is at age 7 taller than 96 per cent and heavier than 91 per cent. Certainly pounds follow the inch in these two boys. It may be said that these are beautiful examples. Beautiful they are, but they are also not far from typical. Records were made of groups averaging 81 in number at each age and all taller than 90 per cent of other boys. It appeared that half of these tall boys varied less than 5.0 grades. Similarly, height and weight were compared in groups averaging 64 in number and all taller than only 10 per cent of other boys. It appeared that half of these short boys varied less than 3.8 grades. Again pounds follow the inch.

At this point arose the difficulty of translating these differences in percentile grades into pounds. An example will make this difficulty clear. It is easy to see that the difference of five grades between the 90th and the 95th percentile grade at age 7 is 2.5 pounds. But the five grades between the 93rd and the 98th have a value of 5.7 pounds, owing to the rapid rise of the weight curve in this region. It is not possible to construct a practicable table showing the value in pounds of all the differences in percentile grades starting at each grade from 90 to 99. It will not be denied that the method of figure 3 is valuable. A comparison of the percentile height and weight, as in figure 3, shows very strikingly that unusually tall boys are also unusually heavy, and that very short boys are almost proportionally light. It provides the foundation for an illuminating dictum: if you know a boys' age and his height, you can usually come within a very few pounds of guessing his weight. But for determining these few pounds more precisely, a second method has seemed desirable.

This second method rests on the fact that if all the boys who are, for example, in the 60th percentile grade in height at age ten (i.e., who are taller than exactly 60 per cent of other boys aged ten), are arranged in a line according to their weights, the lightest boy at one end and the heaviest at the other, the middle boy will be heavier than 60 per cent of other boys aged ten. Table 4 justifies this statement.* To make the table, the weights of boys at each inch of height were recorded, and the 50 percentile grade at each inch of height was calculated for each year of age. Thus at age seven the median boy 47 inches tall weighs 49 pounds. The percentile grade of 47 inches at age seven was

TABLE 4

	AGE									
	5		6		7		8		9	
	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed
inches										
41	37.4	38.0								
42	39.9	39.5	39.0	40.5						
43	42.6	41.5	41.1	41.5						
44	45.2	43.0	43.4	43.5	42.8	43.5				
45	47.5	45.5	45.7	45.5	44.9	45.5				
46			48.0	47.0	47.3	47.5	47.2	47.5		
47			51.0	49.0	49.9	49.0	49.2	50.0		
48					52.5	51.5	51.7	52.0	51.4	52.5
49					55.2	53.5	54.4	53.5	53.9	54.5
50							57.3	56.0	56.8	56.5
51							60.5	58.5	59.8	59.0
52									62.7	62.0
53									66.5	64.5
54									70.2	67.5

	AGE							
	10		11		12		13	
	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed
50	56.2	57.0						
51	59.3	60.0						
52	62.4	62.5	61.8	63.0				
53	65.4	64.5	65.0	65.5				
54	68.3	67.5	68.5	68.5	67.9	69.0		
55	72.3	70.0	71.9	71.5	71.3	72.0	71.9	72.0
56			74.8	73.5	74.6	74.5	75.2	75.0
57			79.1	77.0	78.1	77.5	79.1	78.5
58			84.1	80.5	82.0	80.5	83.3	82.5
59					86.9	84.0	87.2	85.5
60					91.9	88.0	92.1	88.5
61							97.3	94.0

then noted. Boys of this height are in the 48th percentile grade; they are taller than 48 per cent of boys aged seven. Finally, the weight at the 48th grade for age seven was set down. This weight is 49.9 pounds—only nine-tenths of a pound from the observed weight. From this and from other similar cases of agreement it follows that at each inch the median boy is in the same percentile grade in both height and weight. This is again the main conclusion reached by the previous method, that illustrated by figure 3.

Let us now apply this second method to individual boys.

For this purpose two groups of fifty boys each were drawn at random. If the results obtained from one group agreed with those from the other it would be clear that the material was statistically sufficient. Each group was treated in the manner shown in table 5, which gives the result obtained with the first five boys aged six. Eliot T., for example, was

TABLE 5

NAME	HEIGHT	PERCENTILE GRADE	CALCULATED WEIGHT AT THIS GRADE	BOY'S ACTUAL WEIGHT	DIFFERENCE BETWEEN CALCULATED AND OBSERVED WEIGHT
	inches	*per cent*	*pounds*	*pounds*	*pounds*
Israel K.	$43\frac{7}{8}$	30	43.1	45.1	2.0
Joseph M.	$45\frac{3}{8}$	57	46.6	49.2	2.6
John G.	$43\frac{2}{8}$	20	41.7	41.7	0
Joseph C.	$46\frac{6}{8}$	80	50.1	47.9	2.2
Eliot T.	$48\frac{1}{8}$	95	55.1	56.5	1.4

taller than 95 per cent of other boys. The weight of boys who are heavier than 95 per cent of other boys is 55.1 pounds, whereas Eliot T. actually weighed 56.5 pounds. The difference is 1.4 pounds. The process shown in table 5 was carried out for all the boys from ages 6 to 13. The differences between the calculated and observed weights averaged the same in the two groups. At age ten, for example, the median difference was 2.9 pounds in Group I and 3.0 pounds in Group II. The two groups were then joined and the values shown in table 6 were obtained for the hundred boys.

Table 6 states that usually a boy's weight is within a very few pounds of the weight for the same percentile rank as that in which his height is found. At age 6 the difference in half the boys will be less than 2.0 pounds and in three-quarters it will be less than 3.4 pounds.

Again the evidence shows that if we have the inches we shall also usually have the pounds.

In conclusion, therefore, those who have in charge the growth of children should aim first at making their wards taller than the average, and should watch the weight chiefly because a failure to make the normal gain is an index of disease.

TABLE 6

AGE	50 PER CENT	75 PER CENT
	pounds	*pounds*
6	2.0	3.4
7	2.2	3.9
8	2.2	4.0
9	2.8	4.7
10	3.0	6.3
11	3.5	6.0
12	3.5	6.4
13	5.2	8.6

VI

The foregoing statements may be summarized as follows.

1. The rate of growth for an individual child cannot be had by subtracting the average weights or heights of two succeeding years. The difference thus obtained is one year's increase in an average; it is a statistical and not a personal growth.

2. The present investigation determines the growth of the individual boy in relation to the growth of the average. It gives the limits within which the former standards—expressing statistical growth—may be used in prescribing for the individual child.

3. Approximately one-third of the boys do not leave their percentile rank in either weight or height in any one year; the chances are even that no boy will deviate more than 2.7 percentile grades; the chances are three to one that no boy will deviate as much as 5.3 grades.

4. Deviations in height are closely similar to those in weight.

5. Small boys tend to remain small unless rescued by a wise adviser.

6. For determining the monthly growth in weight, the tables already in use need to be corrected only for seasonal variation; monthly measurements do not need to be corrected for personal deviation.

7. The monthly growth in height is too small to require correction for personal deviation, but such correction should be made for observations including three months or more.

8. A failure to make the normal gain in weight is an invitation to disease or the evidence of disease already present; indeed, a failure to make the normal gain in weight is in itself a pathological state.

9. Aside from its use in the recognition of approaching or present illness, the gain in weight is not our chief care; our chief duty is to make our children reasonably tall; the parental and social incompetence which produces stunted children does not repair its fault by making the pounds of a defective boy bear the customary ratio to his defective inches.

10. Usually a boy's weight is within a very few pounds of the weight for the same percentile rank as that in which his height is found.

11. Except in sick children, if we get the inches, the pounds will look after themselves.

THE EFFECT OF ADRENALECTOMY UPON THE TOTAL METABOLISM OF THE CAT[1]

JOSEPH C. AUB, JONATHAN FORMAN AND ELIZABETH M. BRIGHT

From the Laboratories of Physiology of the Harvard Medical School

Received for publication May 9, 1922

One of the most important functions of the glands of internal secretion is to regulate the speed of metabolism of the whole organism. The thyroid gland has long been recognized as the chief factor in this regulatory mechanism—for its removal causes a drop in the normal metabolic rate by as much as 40 per cent, and over-secretion or its ingestion causes a rise which may reach 125 per cent above the normal.

The thyroid, however, is probably not the only gland which influences this normal rate of oxidation, for there is considerable evidence that other glands play a part. These investigations have been made largely by studying the results of the removal of various organs in the surviving animal, as well as the effects of administration of the glands themselves or of their extracts. This paper deals with the basal metabolic changes which follow the extirpation of the adrenal glands. Only a short summary of the recent literature of allied studies will be given here in order to recall the present status of the problem, for a more thorough review will be undertaken elsewhere (1).

Literature. The anterior pituitary apparently plays some rôle in metabolism, as is shown by Benedict and Homans (2). They removed the anterior lobe of the pituitary gland in the dog and followed the metabolism with great care for many months. They noted that with the deposit of fat, which began about a month after the operation, there developed a drop in the carbon dioxide production per kilo of body weight. The fall of metabolism was about 10 to 35 per cent. Perhaps this may be explained in part by the length of the experiment and the immaturity of the dogs at the beginning of the study. This fall of metabolism did not occur until a month after operation and therefore is of slow onset, and may be due to inter-relationship of the glands of

[1] Preliminary Report in the Transactions of the American Physiological Society, This Journal, 1921, lv, 293.

internal secretion. Changes have also been observed in man with abnormal pituitary function. Magnus Levy (3) found an increased metabolism in acromegaly; but he thought this could be explained by abnormalities in other glands. Means (4) has found a reduced metabolism in cases of hypopituitary disease associated with obesity. Boothby (5), however, in a recent note based on wide experience with humans remarked that there is "as yet little evidence that the secretion of any part of the pituitary gland is concerned with the normal rate of cellular combustion in the sense that it acts as a calorigenetic agent." Recent work by McKinlay (6) showed that injection of 1 cc. of a solution of posterior lobe of the pituitary gland increased the metabolic rate in normal individuals about 5 per cent, and lowered the rate in myxedematous patients. This may be suggestive work, but the variations are not sufficiently large to justify accepting only one observation for the normal basal value.

These investigations suggest that the pituitary gland may influence metabolism,—over-secretion causing an increase, under-secretion a decrease. This change in metabolism is not large, and not certain, and its onset does not occur for some time after the removal of the gland.

That the gonads play some part in the regulation of the basal metabolic rate was first suggested by Loewy and Richter (7) who studied a male and a female dog before and after castration. They found that there was very little drop in metabolism three weeks after operation, but that three months later the female dog showed a fall of 18 per cent in the rate of metabolism per kilo body weight. Murlin and Bailey (8) have confirmed this work, but Luethje (9), using different animals for his controls, could find no change in metabolism. Leo Zuntz (10) studied four women before and after removal of the ovaries and found that the drop in the rate after operation was not constant, and did not occur for some time after the operation. Korentchewsky (11) thinks this fall in metabolism following castration is due to a synergistic action of the thyroid,—because Rovinsky (12) found that in thyroidectomized animals the metabolic effects of castration were very slight.

That the spleen is a factor in this mechanism has recently been suggested by Danoff (13). After removal of the spleen of four white rats he found the metabolism rose progressively until, shortly before death, it reached 50 per cent above normal. The animals died within ten days after the operation. Because of this early death, and because no indication of body temperature is given, this work can hardly be

used as a basis of generalization in the case of animals which survive indefinitely after splenectomy. Korentchewsky (14) studied the effects of removing the spleen in three dogs and was unable to find any marked effect upon the gaseous metabolism. While the matter is therefore not settled it seems probable that the spleen has very little effect upon metabolism.

Work by Kojima (15) on white rats suggests that parathyroidectomy plays no part in the regulation of the metabolic rate. Recent work by Ruchti (16) indicates that removal of the thymus gland of rabbits does not affect the respiratory metabolism.

To summarize the literature on these various glands, we find that removal of the anterior pituitary and of the gonads causes, after several weeks, a slight reduction of the basal metabolic rate.

The first good work that has been published upon the relationship of the adrenal glands to the metabolic rate was that reported by Gradinescu (17). He removed the adrenal glands of one dog and three cats, the longest survivals being but 36 and 53 hours. During this time the temperature of the animals was falling, reaching as low a level as 34, 30 and 24°C. It is not mentioned whether the animals were active in the box before operation or whether they were fed at any time during the experiment; nor is there any investigation of their blood pressure. In all cases, however, a marked drop of metabolism is shown following adrenalectomy which runs quite parallel with the fall in body temperature. Gradinescu attributes this fall to a lack of regulatory mechanism for the peripheral vessels, whose movements are normally controlled by the adrenin from the suprarenal capsules. This work is to be criticised on the grounds already spoken of, but most particularly because of the fall of body temperature and the question of activity during the observations. Marine and Baumann (18) have published the interesting results of their studies on the metabolism following adrenalectomy in rabbits. Their work and the contents of our present paper were both first reported at the same meetings. Very recently and since our paper was written they have published another article on the interrelation of the adrenal and thyroid glands (19). Briefly, their findings are that in rabbits there is a preliminary fall in metabolism after adrenalectomy, followed by either a rise, a fall or no change from the original rate of metabolism. In most cases, however, there is a distinct increase in the metabolic rate "followed by a fall to or below the normal, whether dying within 2 or 3 weeks or living on indefinitely." The average maximum rise for the group was 23 per cent. In the sec-

ond paper experiments are reported in which adrenalectomy was done on six thyroidectomized rabbits, and in these animals only one showed a rise in the metabolic rate. From this the authors conclude that the adrenal effect on metabolism is largely due to an antagonism between the adrenal cortex and the thyroid, the increased metabolism being developed through the thyroid when the depressing influence of the cortex is removed. These experiments and ours differ essentially in that Marine and Baumann find an intermediate rise of metabolism occurring in their surviving rabbits. We have tried using rabbits and have found it difficult to determine true basal metabolism on them because of their activity. Marine and Baumann have not determined activity, and where data are derived from single unchecked periods of two hours it seems highly probable that activity must be a definite factor in the results. This is clearly shown in their remark that with the rise in metabolism their animals were "more active, more restless, more easily irritated and sometimes became vicious." They have also not reported the body temperature. Furthermore, the very high respiratory quotients suggest that their animals were not in a thoroughly basal condition. We have not noted the intermediate rise of metabolism in partially adrenalectomized cats, and the few cases of Addison's disease to be found in man have shown a reduced metabolism. The striking difference between the work of Marine and Baumann and of ourselves lies in the interpretation of the data at hand, and it is only with the accumulation of further observations that the true relationship between the thyroid and the adrenals will be thoroughly established.

The work here reported was undertaken with the object of studying more thoroughly the possible independent relationship of the adrenal glands to the control of the metabolic rate. An attempt was made to keep the cats in excellent condition, with normal body temperature and normal blood pressure, and to control all abnormal factors which developed during operation, in order to determine whether the mere removal of the secretion of the adrenal glands would affect the metabolic rate of the organism.

Methods. The method used for determining the basal metabolic rate of the cat is in all essentials that described by Benedict and Homans (2). A small copper box of 19 liters capacity is placed in a larger box which is water jacketed so that the temperature is kept almost constant during a given experiment. The copper box in which the animal is placed is made air-tight throughout by sealing all connections by water cups. It is connected with a Benedict universal respira-

tion apparatus absorber system. A pump forces the air through the system at a rate of about 17 liters per minute. This circulating air is forced through two bottles of sulphuric acid which removes all moisture; then through a bottle of soda lime and one of sulphuric acid which take out the carbon dioxid, and is then returned to the box. There are two CO_2 absorber systems so that the experiment progresses continuously, and the CO_2 eliminated is determined by weight at the end of each period. A spirometer attached to the system is the only portion of the apparatus which allows for changes in volume. As the volume decreases pure oxygen is admitted through a Bohr meter and the volume absorbed in any one period is thereby estimated. The moisture remaining in the box at the end of each period is estimated by means of psychrometer thermometers. The carbon dioxid remaining may be ignored because of the small volume of the box and the relatively rapid circulation of air. Changes in the volume of the air in the closed system are calculated at the end of each period by correcting for temperature, barometer, and aqueous vapor changes and this correction is made upon the oxygen admitted in order to determine the accurate amount of oxygen absorbed by the cat. By this method the carbon dioxid eliminated and the oxygen utilized by the animal can be accurately determined, and from these figures the rate of metabolism can easily be calculated.

The accuracy of the apparatus was repeatedly studied by alcohol checks which had an average respiratory quotient of 0.66. No observations were made unless these checks were satisfactory and the box shown to be tight by repeated blank runs.

In order to determine the activity of the animal the copper box rested upon a delicate recording apparatus which graphically recorded any movements. This apparatus is similar to that described by Benedict and Homans (2) and consists of a frame, one side of which rests on knife edges, while the other is supported by a wire spring. A pneumograph, connected by air connection with a tambour, records on a kymograph marked oscillations whenever there are slight movements of the animal. Whenever the animal was even slightly restless during a period of observation, that period was eliminated and not counted in our results.

The periods were about 30 minutes each, and whenever possible four quiet periods were obtained in each observation. The animals used for this work were very quiet, docile, full-grown cats which lay very quietly and contentedly in the box. Many normal control ob-

servations were made, just prior to operation, and only the very quiet periods were used in the average control figures. The animals were in the post-absorptive state, no food being allowed for at least 18 hours before the observations. This was true except after double adrenalectomy,—when milk was offered to the animals after each observation. They drank a little during the first two days; after that food and water were generally refused and the animals were fed by stomach tube. The actual amount of milk retained is difficult to estimate as the animals often regurgitated after being fed.

The operations were usually performed in one stage by the posterior route. Ether was used as anesthetic and particular care was taken to keep the animal warm. The adrenalectomy experiments were done largely during the summer when the surroundings were constantly warm. The animals were trained before operation to sit on an electric heating pad and this was kept constantly heated after the operation, and the cats sat on it when they desired warmth. By this means their own body temperature was maintained at a level approximately the same as before the operation; at first at a slightly higher level, but usually falling somewhat as the animal grew weaker. The rather long duration of life in the adrenalectomized animals may be partly accounted for by this maintenance of the normal body temperature.

Urinary nitrogen was not studied because every extra manipulation of the animals was scrupulously avoided. The work of Marshall and Davis (20) shows very clearly that urinary excretion is not an accurate index of protein metabolism after adrenalectomy because there is a retention of urea nitrogen in the blood.

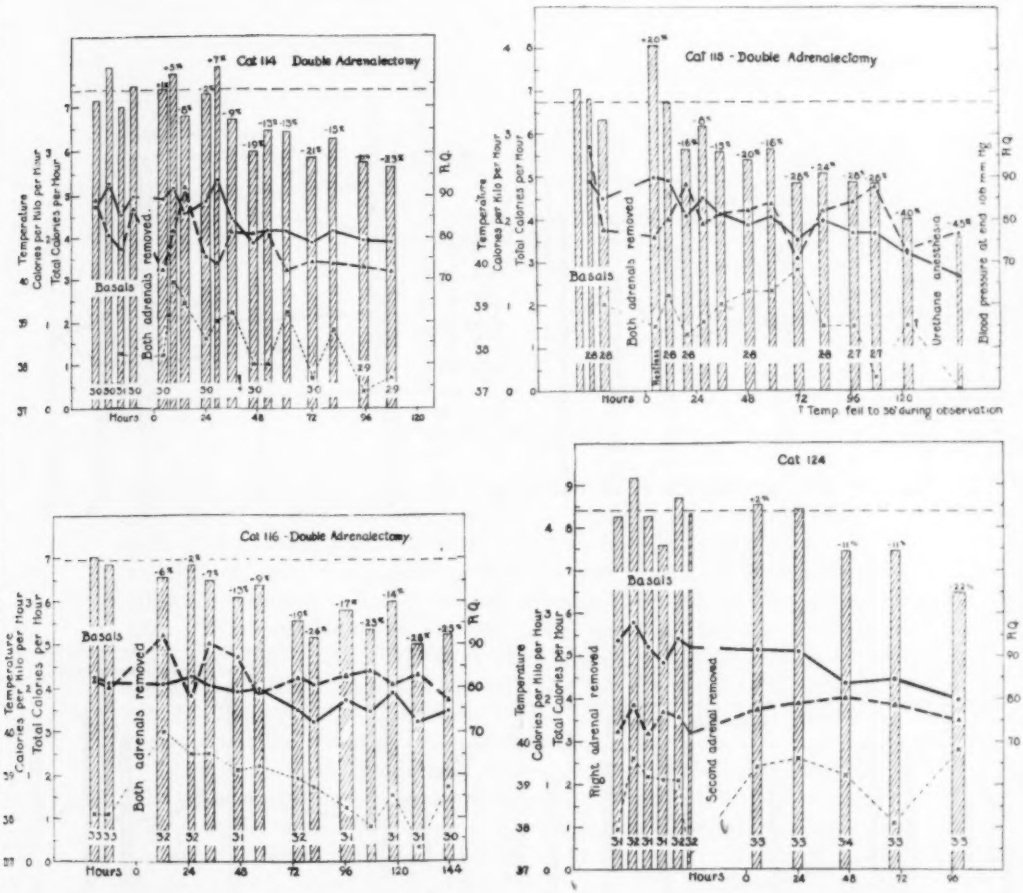
The problem of accessory adrenal tissue left after adrenalectomy is one which must constantly be borne in mind. We have done extremely careful, complete autopsies on all of our animals and in only one (cat 114) was an accessory adrenal found. This measured 3 by 3.5 mm. and was composed almost entirely of cortical tissue. In the center was a thin strand of connective tissue in which there appears to be a rare chromaffine cell. In spite of this tissue there was apparently a distinct adrenal insufficiency with all of the usual signs seen in the other completely operated animals. This animal died with convulsions, but we have seen this occur in several experiments and it is also described by Crowe and Wislocki (21). The accessory adrenal tissue which lies about the nerve ganglia near the abdominal aorta when studied microscopically has often shown an almost complete absence of cells containing chromaffine granules, but in cat 116 some cells were found

stained with Orth's fluid. The enlargement of the abdominal lymph nodes and thymus glands, described by Crowe and Wislocki, was very strikingly shown in our adrenalectomized cats.

General considerations. The tables giving the actual figures of our results are not reproduced because of the large volume of data. The charts show graphically the effects of the various procedures upon the metabolic rate in the longer experiments. Animals that died soon after the operation were discarded. The experiments in which double adrenalectomy was done (cats 114, 115, 116 and 124) show no marked drop in metabolism for about 24 to 48 hours after the operation, and then a rather rapid fall to a level approximately 25 per cent below the average normal. This fall comes at about the time the animals show the onset of weakness, but it is not at all the accompaniment of collapse, for they still walked about the room, jumped in and out of the sink, and were capable of considerable muscular effort of short duration. They did, however, appear incapable of prolonged activity, and after short exertion would stand and wag their tails as though puzzled or would lie down and rest. This was their condition up to the end of the experiment and at no time did they give any evidence of suffering pain. No observations were made of the rate of the metabolism during the final collapse, as it was thought that the results must be complicated by the effects of the low blood pressure. Aub (22) has shown that low blood pressure and shock will cause a drop in metabolism, but the blood pressure of 106 mm. Hg. found in cat 115 at the end of the experiment eliminates this as an explanation of the reduced metabolism after adrenalectomy. Elliott (23) has shown that blood pressure falls when the collapse develops, and Marshall and Davis (20) also mention that the blood pressure remains above a shock level before the animals collapse.

With the fall of total metabolism there is also a fall of the rate when calculated per kilo of body weight. This is not as great as the percentage reduction in the total calories used, but is more striking when compared with the control figures in which practically no fall per kilo of body weight occurs.

There are five factors in these experiments which must be eliminated before this fall in rate may be established. The maintenance of temperature, blood pressure and the usual requirements of inactivity have already been discussed. The two other factors are the effects of diminished food and of operation. In order to control thoroughly the diminished food intake, several animals were allowed to fast completely.



Group I. The effect of double adrenalectomy upon the basal metabolism of the cat.

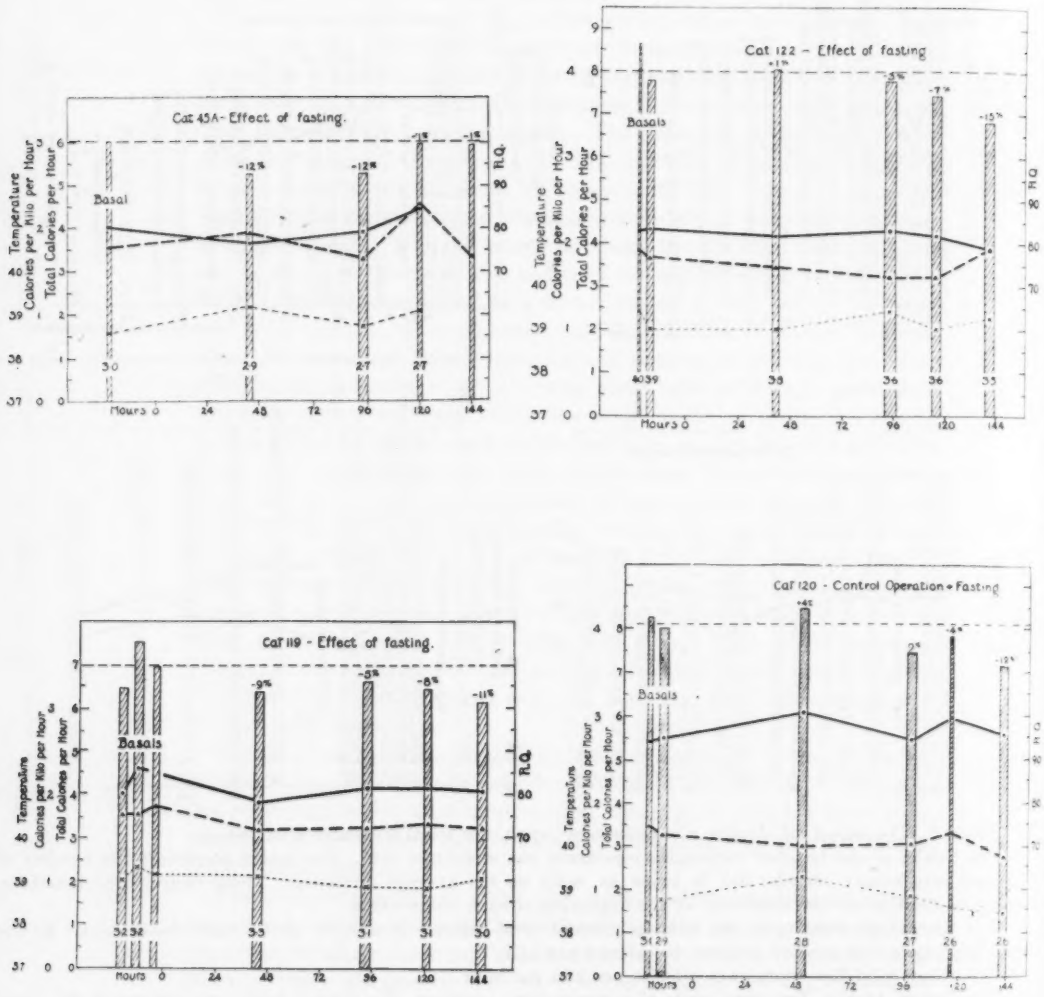
The height of the hatched rectangles represents the metabolic rate. The width represents the number of averaged satisfactory periods, and is twice as wide as the average duration. These figures are, therefore, correct in relation to the time only at the beginning of each observation.

The percentage drop from the average normal total calories is written above each observation. In the preceding paper this number referred to calories per kilo.

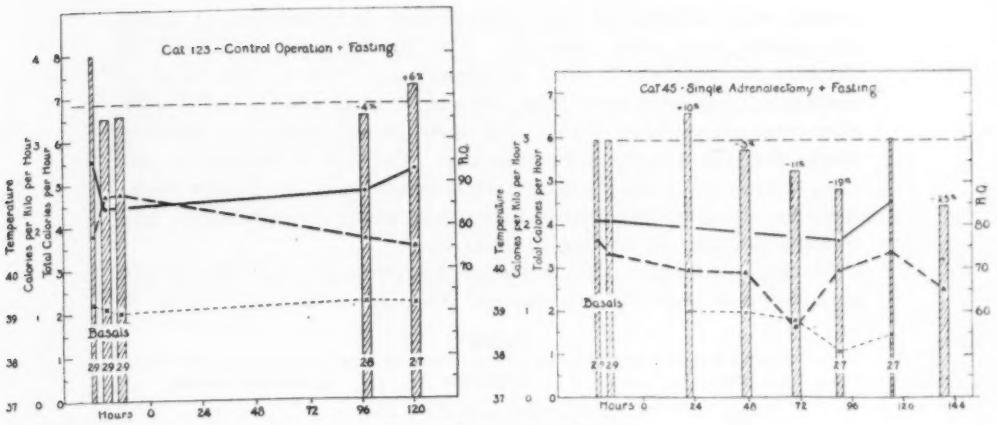
The heavy solid line represents calories per kilo per hour.

The heavy broken line represents the respiratory quotient, the lighter broken line showing the temperature of the animal in centigrade degrees.

The small figures near the bottom of the columns show the weight of the animal at the time of the observations.


Cat 45A - Effect of fasting.
Cat 122 - Effect of fasting
Cat 119 - Effect of fasting.
Cat 120 - Control Operation + Fasting
Basals
Temperature
Calories per Kilo per Hour
Total Calories per Hour
R.Q.
Hours 0 24 48 72 96 120 144

Group II. The effect of fasting and control operation upon the basal metabolism of the cat.



Group II (continued).

This was not the case with the adrenalectomized animals, for cat 114 took 80 cc. of milk, cat 118 took 475 cc., and cat 116 took 560 cc. of milk in the period after operation. In the controls, (exper. 45 A, 119 and 122) in spite of complete fasting, the metabolism fell far less than after adrenalectomy, and there was practically no fall in calories per kilo. These results agree approximately with the findings of Voit (24), and Hari (25). The effect of combining complete fasting and control operations (expers. 120 and 123) was the same as of fasting alone. The operation was similar to that followed in doing an adrenalectomy, but the adrenals were not disturbed.

In experiment 45 one adrenal was removed and the animal fasted. The effect on the metabolism was more marked than in the other controls, but the animal showed signs of adrenal insufficiency in his lack of hunger, relaxation, weakness and fall in temperature. Removal of a single adrenal, but with continuation of normal diet produced a similar fall in the rate (expers. 209 and 210) where, after a preliminary fall of 19 and 20 per cent in metabolism the rate returned to its original level a week after operation. It therefore seems clear that the removal of one adrenal causes a temporary slight fall of metabolism. Removal of the adrenals in two stages gave results similar to those in which a single operation was performed. Cat 124 had one adrenal removed before any observations were made upon it. The removal of the second

adrenal was followed by the usual decline of metabolism at about the normal time after operation. Cat 204 in paper I showed no decrease in metabolism after adrenalectomy. Up to the time of operation it had been given large doses of thyroxin and the well-known slow cumulative effects of this drug upon the metabolic rate probably masks the effects of the adrenalectomy. The lack of reaction in this case, therefore, is probably of very little significance, but merely represents the antagonistic effects of thyroxin in raising the metabolism, and of adrenalectomy in tending to lower it.

Marshall and Davis (20) arrive at the conclusion that the protein metabolism is probably not changed after adrenalectomy. It is, of

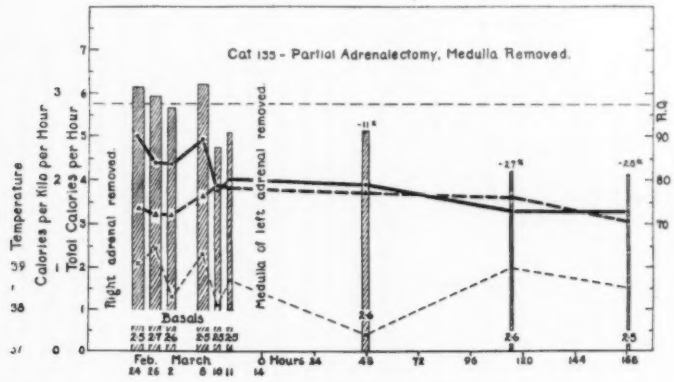
TABLE 1*

DATE	BLOOD UREA	URINARY NITROGEN
1918		
November 18		1.16
November 19		
November 20	38	1.06
Second operation (second adrenal removed)		
November 21		0.70
November 22	95	0.93
November 23		0.66
November 24	92	0.94
November 25		0.86
November 26	105	1.34
November 27	165	0.23

* Taken from Marshall and Davis.

course, difficult to estimate the rate of protein metabolism when there is accumulation of end products such as urea in the blood as well as excretion in the urine. In one case, however, (table IV, cat B 29 of their series) the urea content of the blood remains constant from November 22 to 24, and it is, therefore, fair to assume that the urea produced during this period was excreted. During these two days the average of urinary nitrogen is 28 per cent lower than before adrenalectomy, or 16 per cent if the larger figure alone is taken (table 1). This drop is of about the same magnitude as the fall in metabolism in the adrenalectomy experiments here reported, and is suggestive evidence of a fall in protein metabolism, although it is, of course, but a single observation.

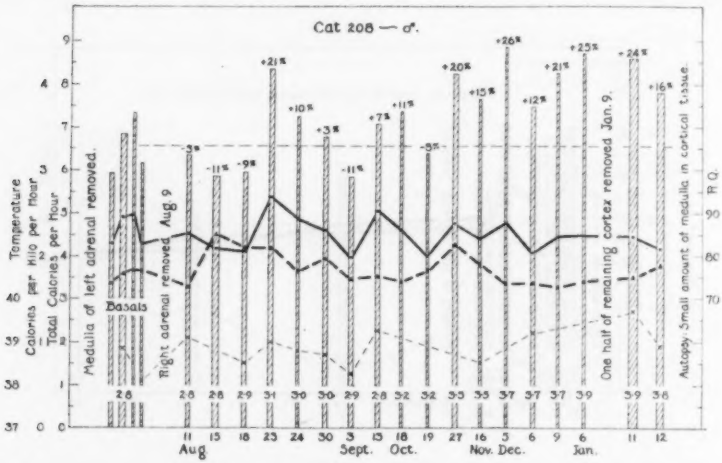
The respiratory quotients were not markedly affected by the operation or the removal of the adrenals. Because no urinary and blood studies were undertaken in these animals, it is not certain that the percentage of foodstuffs burned was the same before and after adrenalectomy. The one observation of Marshall and Davis already discussed, however, makes the deduction extremely probable, that, at most, there would have been but little change in the relative proportion of carbohydrate, fat and protein catabolized. There is, then, associated with the weakness following the removal of both adrenal glands, simply a fall in the rate of metabolism without a marked change in its character.



Many attempts were made to establish the relationship of the two parts of the adrenal to this metabolic change. Experiments were repeatedly performed in which the attempt was made to remove surgically all the medullary tissue, and leave behind only the part supposed to be essential for life, i.e., the cortex. We have never been able completely to accomplish this in cats, for they have nearly all died within 10 days with signs of adrenal insufficiency. Many types of surgical procedure have been tried without success. Experiment 133 is a good example of this series. The first adrenal was completely removed two weeks before any metabolic observations were made. After basal respiration observations were completed, the remaining adrenal was exposed and part of it, which included about one quarter of the cortex and all of the medulla, was cut away. The cat lived eight days after

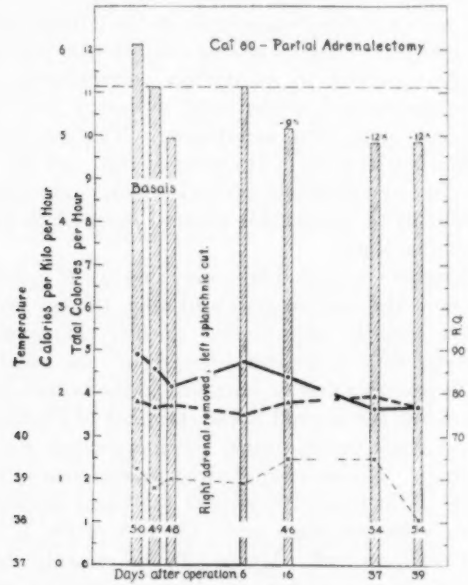
this operation, but died with all signs of adrenal insufficiency. The metabolism fell during this period. The microscopic sections of the adrenal cortex left in the animal were examined by Dr. S. B. Wolbach. He found no recognizable adrenal medulla present, the cortical cells looked normal, with apparently normal vacuolation, no cellular reaction, and, in parts at least, a blood supply that looked intact.

Cat 208 was studied for seven months. Before the metabolic determinations were started, a large part of the left adrenal was removed and the remaining medulla thoroughly scarified. After basal determinations were made, the right adrenal was completely removed, and



the metabolism studied repeatedly during the next five months. The metabolism *per kilo of body weight* showed a slight drop for the week after operation of about 10 per cent, and then a slight rise above normal which was not regularly present during the next two weeks. On the whole, however, one is forced to conclude that the metabolism per kilo remained essentially constant throughout. During this time the animal gained much weight and grew very lazy, although it was out of doors in a large runway. On January 9 over half of the remaining cortical tissue was removed without any effect upon the metabolism during the next three days. Unfortunately the animal died. At autopsy some actively functioning medullary tissue was found imbedded in the remaining cortical tissue.

In order to avoid surgical trauma on the remaining adrenal, a different type of experiment was tried. The right adrenal gland of cat 80 was removed, and the left denervated by section of the splanchnic nerves. The animal, therefore, had no nervous control of this organ, and under these conditions Stewart and Rogoff (26) have shown that the flow of adrenin is reduced to "a small fraction of the normal." The effect upon the cortex of the adrenal should theoretically be slight, as microscopically the nerve supply is almost wholly distributed to the medullary



tissue. The result would therefore tend to show the effects of diminished secretion of adrenin rather than change in the cortex. Sixteen days after operation the metabolism had fallen slightly, with the weight of the animal reduced from 4.75 kgm. to 4.62 kgm. This is quite different from the extirpation experiments where the reduction of metabolism occurred two days after removal of the adrenals. Thirty-seven days after operation the weight of the animal had risen to 5.36 kgm., and it was in excellent condition, but the total metabolism had fallen 12 per cent and per kilo it had fallen from the normal value of 2.27 calories to 1.85 calories per kilo per hour, which is a reduction of 19 per cent.

We find, then, a reduction of the metabolic rate both in total heat production and calories per kilo in spite of a decided increase in weight. Whether this deposit of fat would normally cause an increase of metabolism cannot now be definitely stated, though work on the obese by Means (4) suggests that it would. One might expect that this fat deposit would be inert tissue, the presence of which would demand no increased heat production from the organism, but even if this be true the total metabolism, quite independent of weight, was also reduced in this experiment. Here, then, we have an animal in excellent physical condition, with a diminished activity of the adrenal medulla, which showed a distinct fall in the metabolic rate, though not so rapid or so great as those observed in completely adrenalectomized animals.

In the adrenalectomized animal with accessory cortical tissue (cat 114) there was a typical fall in metabolism. This cat died of apparent adrenal insufficiency in spite of the cortical rest, and the fall in metabolism was similar to completely adrenalectomized animals. However, it would be difficult to ascribe this reduced metabolism to the absence of adrenal medulla alone.

It cannot definitely be stated here that this fall of metabolism is also seen in diseases of the adrenal glands in man, though several such observations have recently been made. Loeffler (27) reports a case of Addison's disease with a distinct lowering of the basal metabolism. A woman of 39 years showed on entrance to the hospital a metabolism 26.5 per cent below the normal linear standard of DuBois (28). Two months later, a week before death, the metabolism was 20 per cent below the normal. The autopsy showed tuberculosis of both adrenals with caseation. Muirhead (29) reports the basal metabolic rate in a case of Addison's disease, when under the care of Dr. L. G. Rowntree, as 30 per cent below normal. Means (30) has recently observed a mild case of Addison's disease with a rate 15 per cent below normal. Case 7032 at the Peter Bent Brigham Hospital showed a metabolism 12 per cent below the average normal in spite of being "restless during entire experiment." At autopsy the adrenals in this case showed caseous tubercular nodules with only a few strands of cortical adrenal cells intact.[2]

The fall of metabolic rate here described is quite different from that seen after removing other glands. After removal of the hypophysis or the gonads, definite changes did not occur for weeks or months.

[2] We wish to express our thanks to Doctors Christian and Sturgis and to Doctor Means for permission to use this evidence.

Three days after thyroidectomy Rovinsky (12) found the oxygen consumption in rabbits reduced by only 8 per cent in two animals and 21 per cent in another, and in one dog a fall of only 6 per cent. Marine and Lenhart (31) also have recorded the effect on metabolism three days after thyroidectomy in one rabbit. The metabolism rose 5 per cent in this instance. With complete removal of the thyroid, therefore, the fall in metabolism is less rapid than after adrenalectomy, when the fall is quite prompt in onset and fairly abrupt in reaching a lower level, and then tending to maintain that level. This fact, together with evidence shown in the accompanying papers (32) strongly suggests that the fall in metabolism after adrenalectomy is an independent mechanism, and not due to effects upon interrelated glands, such as the thyroid. In support of this is the following further evidence: *a*, Under anesthetics the lowered metabolic rate due to adrenalectomy may be returned to normal by intravenous injection of physiological amounts of adrenalin (33), an experiment which supports the view that the fall of metabolism is due to lack of adrenin itself. *b*, Adrenalectomy done under urethane anesthesia caused a drop in metabolism even though the thyroid gland had previously been removed (33). *c*, Adrenin injections cause an increased metabolism in rabbits even though they have been deprived of their thyroid glands (31). We have been unable to confirm this fact in cats, because of the marked activity which follows adrenalin injections. Lusk (34) had the same difficulty with dogs. *d*, In animals made thyrotoxic by the injection of thyroxin, removal of the adrenals does not cause a return of the raised metabolism to its original level (32). *e*, Very recently Martin and Armitstead (35) have shown that the metabolism of the resting isolated frog muscle is greatly increased when exposed to adrenalin solutions and Evans (36) has shown that the isolated heart also has a markedly increased metabolism and pulse rate when adrenalin is perfused.

These facts point definitely to the conclusions, first, that the thyroid gland is not necessary for the stimulating action of adrenin or the depressing effect of adrenalectomy under anesthesia, and secondly, that the adrenals are not necessary for the stimulating effect of thyroxin. The two mechanisms may, therefore, be distinct. It is clearly established that the action of adrenin is one of very rapid onset, and of duration which may be measured in minutes (37). The action of thyroxin, on the contrary, takes many hours to become established, and then lasts for days, as has been shown by studies at the Mayo Clinic (38).

The theory, therefore, is suggested that here are two mechanisms which may function independently. The adrenal glands, probably by means of adrenin, may help in the acute chemical regulation of metabolism and may be the basis of the increased rate seen during excitement. The slower acting regulation would then be accomplished normally by the thyroid secretion. This is analogous to the mechanism of the body for maintaining a normal hydrogen ion concentration, where elimination of CO_2 by the lungs is the acute, rather rough method, but elimination of phosphates by the kidney is the slower, more accurate regulator. This point of view would give us a possible explanation of the rather high metabolic rates so often seen in excited human subjects. For instance, during the first determinations on a nervous patient the rate of metabolism is often quite high, even though the patient remain very still, and as the excitement wears off the rate rapidly declines. According to the emergency theory of adrenal secretion advanced by Cannon, it would be during this period of excitement that adrenin might be secreted at an increased rate, and so stimulate the metabolism. The maintenance of the normal metabolic rate for the hours immediately after adrenalectomy may be explained by the presence of the accessory chromaffine tissue along the nerve ganglia. Microscopic examination of this tissue shows it to be often depleted of chromaffine granules after adrenalectomy, which is not the case in intact animals. This suggests that it has become largely exhausted of its small adrenin store since the operation.

Much evidence has therefore been brought forward to show the influence of the adrenal glands upon the basal metabolism. This effect apparently is different from that exerted by any other organ in the body in that the effects develop rather suddenly and markedly a few hours after adrenalectomy and the effects of adrenalin injection are marked but of short duration. There is evidence against this being due to interrelationship of glands, which points to the adrenals as being an independent mechanism for the regulation of acute metabolic changes.

CONCLUSIONS

Forty-eight hours after the removal of both adrenal glands in the cat there is a reduction of about 25 per cent in the total metabolism without marked change in the relative percentages of foodstuffs burned.

This drop in the metabolic rate is evident when measured as total calories, or calories per kilo of body weight.

Control experiments on completely fasting and operated cats show a fall in metabolism less than half the magnitude of that seen after adrenalectomy.

The removal of one adrenal causes a temporary fall, but a return to normal metabolism. Denervation of the remaining adrenal is followed by a slow fall of the metabolic rate.

Several human cases of Addison's disease, collected from other sources, showed a reduced metabolic rate.

Evidence for the independence of the thyroid and adrenal effects upon the metabolic rate is discussed.

The theory is suggested that whereas the thyroid is the slowly acting regulator of the metabolic mechanism, some quick changes of short duration may be controlled by variations in the secretion of the adrenal glands.

SOME ABBREVIATED PROTOCOLS

Cat 114. Male. Weight 3.0 kgm. Normal temperature 38.3°C. Double adrenalectomy.

Many normal control periods were obtained.

May 20, 1920. 9:30 to 11:00. Both adrenal glands were removed aseptically by the posterior route. The temperature at the end of the operation was 36.1°C. The animal was put in the incubator room after the operation.

12:35. The metabolism determination was started, the chamber having been warmed previously with electric lights. At the end of the run the cat's temperature was 39.6°C. Pulse 200. The cat was quite well until

May 22, when he showed some weakness, but could still jump in and out of the sink easily. He drank milk. The legs were spread apart when standing.

May 23. 12:15. Many red blood cells in the urine. The cat did not walk about much, had developed a cough and his nose was obstructed with mucus.

5:15. He jumped normally into the sink. Appetite diminished. Defecations well formed.

May 24. 8:00 p.m. Cat had become noticeably weaker, walking with difficulty and reluctantly, and lying flat on the heating pad.

May 25. 11:00 a.m. Very weak. Was fed by stomach tube; this caused a convulsion and the animal died,—just 5 days after operation.

An autopsy was performed immediately. The positive findings were: Trachea blocked at bifurcation by a mucus plug. Right kidney showed a fresh blood clot, 1 cm. in diameter, confined entirely to subcapsular region.

At the junction of the left renal vein with the vena cava was found a small ovoid body 3.0 by 3.5 mm. in diameter. The tissue was firm, dull yellow in color with a reddish central area. Microscopic section after fixation in 2 per cent bichromate formalin solution showed this to be adrenal cortex except for a very thin central strand of connective tissue in which a few medullary cells appeared.

The paraganglion tissue gave no chromaffine reaction when stained in bichromate solution. Microscopic section of nodes from the abdominal sympathetic chain showed no chromaffine staining tissue.

Cat 115. Female. Weight 2.8 kgm. Average temperature 38.4°C. Double adrenalectomy.

Normal control periods obtained May 27 and 28, and June 4, 5 and 7, 1920. Cat in good condition.

June 9. 10:21 to 11:19 a.m. Both adrenal glands were removed aseptically by the posterior route. No blood lost. After operation animal was put in warm room to keep temperature normal.

1:20. Cat completely recovered and purring contentedly.

June 10, 11 and 12. Cat appeared quite normal, maintaining normal weight and temperature. Drank milk and water freely.

June 13. Appearance of transient signs of weakness as manifested by slight tremor of the hind legs. Given milk by stomach tube.

June 14. Cat became progressively weaker during the day. Walked without stopping but swayed when standing still.

2:38 p.m. Anesthetized animal with 25 per cent urethane,—123 hours after operation. Blood pressure cannula rapidly inserted. Blood pressure 106 mm. Hg. Obtained two short metabolism periods before blood pressure fell to the shock level of 80 mm. Hg.

Autopsy showed: Adrenalectomy complete. No accessory adrenals.

Cat 116. Male. Weight 3.3 kgm. Double adrenalectomy. Good basal observations were made on May 26, 27, 28 and June 23, 24, 1920.

June 25. 10:45 a.m. Adrenalectomy was performed aseptically under ether anesthesia. Temperature 39.5°C. Pulse 180. Respiration 148. The cat remained quite well, drinking milk and showing no marked signs of weakness until on the evening of

June 29, he showed distinct signs of weakness. He swayed slightly when walking, and the hind legs appeared stiff.

July 1. The cat was quite weak, lay on his side fully extended, and would walk only a few steps at a time. Weight 3.0 kgm. Temperature 39.2°C. Pulse 228. Respiration 356. After considerable difficulty a stomach tube was passed; 147 hours after adrenalectomy urethane was given, but only after a struggle, and anesthetization did not take place for a long time. As soon as the blood pressure could be recorded it was found to have dropped to 70 mm. Hg. and the experiment was discontinued.

The positive findings at autopsy were: Mesenteric and retroperitoneal lymph nodes enlarged. Slight chromaffine reaction in the paraganglionic tissue after fixation in Orth's fluid. No accessory adrenal tissue.

Cat 124. Male. Removal of one adrenal medulla, followed by removal of the other adrenal which was found to be much hypertrophied.

November 7. The medulla of the left adrenal was entirely removed. For a day the cat was very sick, probably with peritonitis, but without visible signs of sepsis, the belly remaining soft and not distended or rigid.

January 18 to February 13. The determinations of the basal metabolism were made.

February 14. The right adrenal gland, which was markedly hypertrophied, was removed.

February 15 and 16. The cat had recovered so that he drank milk and ate meat.

February 17. He grew gradually weaker and preferred to sit on a soft pad.

February 18. Adrenal lipoids of the ox made up in salt solution were given intramuscularly. The metabolism was low.

February 19. Cat extremely weak and had to try several times to assume a standing position. He was given Klim by stomach tube, but promptly vomited it all. There was constant and frequently violent twitching of the fore and hind limbs and at times of the whole body. He died at noon.

At autopsy there was no evidence of sepsis. All the organs looked normal. Both adrenals were absent. In the left adrenal area, from which the medulla had been removed, there was a clean scar without peritoneal adhesions and without even the slightest trace of adrenal tissue. Several pieces of fat when examined microscopically showed no cortical tissue. The right adrenal area was clean, no adrenal tissue being present. No accessory adrenal tissue was found.

Scattered throughout the lungs there were about 7 round hemorrhagic areas which floated and suggested recent infarcts. No thymus tissue was seen. Microscopic section of the pituitary gland showed that there was no abnormality of structure or granules. The animal apparently died of adrenal insufficiency.

Cat 80. Male. Weight 4.8 kgm. Basal metabolic determinations were obtained on March 11, 15, 17, 18, 1920.

March 19. The right adrenal was removed and the left splanchnic nerves were cut by Dr. W. B. Cannon. The animal recovered rapidly.

March 25, April 4, 25 and 27. The metabolism was determined, the cat being in excellent condition but very fat and lazy.

March 27. He was given 0.27 mgm. caffeine intramuscularly.

March 28. Transperitoneal medullectomy of the left side was performed. At the end of the operation the cat was in good condition, with satisfactory pulse and appearance.

6:00 p. m. He was restless. Respiration normal. Pulse 240. Cat was nauseated. He died that night, apparently of adrenal insufficiency.

Cat 45. Male. Operation, starvation, adrenalin injection, caffeine injection. Weight 2.9 kgm.

October 16, 18 and 19. Determinations of the basal metabolsm were made.

October 19. 3:00 to 3:40 p.m. The right adrenal was removed aseptically under ether anesthesia. There was very little bleeding. The cat recovered in a warm room and drank a little milk.

October 20. 9:30 a.m. Temperature 39.0°C. The cat was in excellent condition.

October 21. The cat's reactions had changed. He sat quietly with his legs curled under him, his belly flat on the ground. He liked to be petted, showed no desire for food or water, and did not walk about. Temperature 38.9°C.

October 22, to 28. Temperature 38.8°C. The cat ate progressively more and seemed quite well by October 28.

November 4. Weight 3.00 kgm. Temperature 38.55°C. For the past ten days he had taken large quantities of meat and milk.

November 9. Observations were made on the effects of fasting. The animal had had no food for 5 days.

November 21. Since the period of fasting the cat had been eating freely, had lived in the outside cage and appeared to be in good condition. Food was taken away from him at 2:00 p.m.

November 22. Temperature 39.25°C. Weight at 9:00 a.m. was 2.72 kgm. Basal determinations were made.

2:44 p.m. one cubic centimeter of adrenalin was injected intramuscularly. The cat was only slightly excited by the injection.

November 23. Temperature 38.35°C. Weight 2.66 kgm. Basal determinations were made in the morning.

2:05 p.m. Caffeine sodium benzoate was given intramuscularly (0.025 gram caffeine alkaloid per kilo).

November 24. Temperature 38.50°C. Weight 2.61 kgm. The cat was very restless so that several periods had to be rejected. The left adrenal was removed aseptically, but the cat died during the night, apparently from adrenal insufficiency.

Cat 120. Female. Weight 3.0 kgm. Average temperature 38.7°C. Control operation and fasting.

November 11, 12 and 16. Determinations of the basal metabolic rate were made.

November 16. A control operation was performed in which the usual technic of adrenalectomy was followed except that the adrenal blood vessels were not tied and the adrenal glands were not removed. No food was given to the animal until the afternoon of

November 21, when the cat weighed 2.56 kgm. Her temperature was 38.38°C. The wounds were healing well. Fasting lasted for 6 days,—after the operation 116 hours.

Cat 122. Control operation and fasting.

November 30, December 3 and 6. Determinations of the basal metabolic rate were made. Weight 3.1 kgm. Temperature 39.5°C.

December 8. Control operation performed in which incisions were made on both sides exposing the adrenal glands which were left *in situ*. No food was given the animal after the night of December 8 until

December 14. The cat was healthy throughout the period of fasting, but at the end of the period was very hungry. The body weight fell from 3.3 kgm. to 2.87 kgm.

January 4. Animal was well and appeared in excellent condition. Weight 4.02 kgm. Temperature 39.4°C.

January 7. Another period of fasting was begun. Weight 3.95 kgm. Temperature 38.7°C.

January 12. This was the last day of the fast. Weight 3.50 kgm. Temperature 39.25°C.

Cat 119. Female. Weight 3.25 kgm. Temperature 39 to 39.35°C. This was a very quiet, gentle animal.

October 26, 27, 28. The determinations of the basal metabolism were made. After each determination the animal was fed.

November 3. The cat had been given no food since October 28. She was in good condition but very hungry. Temperature 39.25°C. Weight 3.03 kgm.

Cat 208. Male.

July 14. This was a very mild cat in excellent condition. Weight 2.58 kgm. Operation: By an abdominal incision the left adrenal was exposed; its lower outer pole was removed. A probe wet with alcohol was used to scarify the remaining medullary tissue.

August 2. After slow recovery from the operation the cat appeared in excellent condition. The basal metabolism was determined for the first time. Weight 2.73 kgm.

August 4, 8, 9. Satisfactory observations of the basal metabolism were obtained.

August 9. The right adrenal gland was removed under ether anesthesia.

August 15. The cat was still in excellent condition, eating and acting normally. Put in the outside runway.

August 31. The stitches removed from the wound.

September 1. The wound separated a little. Weight 3.02 kgm. Temperature 38.70°C.

September 9. The cat's character has changed; he is very lazy, dislikes to walk about, and is very affectionate.

January 9. The cat had remained perfectly well. The basal metabolism had been repeatedly studied during the past 4 months; many of the observations had to be discarded because of activity. Aseptic operation: The remaining adrenal was found to be markedly hypertrophied. The whole upper pole was removed,—well over half the remaining tissue being taken out without tying any blood supply.

Serial sections of this tissue were studied for us by Prof. S. B. Wolbach who found no medullary cells present, but some markedly hypertrophied cortical tissue. "The deposit in the central portion looks like calcium, and lipoid which probably comes from cortical cells. There is very little connective tissue present."

January 16. The animal had been in excellent condition without evidence of adrenal insufficiency until on this day the wound was found partly opened. The cat was promptly anesthetized, and the exposed omentum was removed.

January 17. The cat died.

Immediate autopsy showed that there was peritonitis, evidently from the open wound. No accessory adrenals were found. The remaining adrenal tissue was very small in amount. On serial section it was found to contain cortical cells for the most part, but also a strand of medullary cells loaded with chromaffine staining granules.

BIBLIOGRAPHY

(1) Aub and Taylor: Endocrinol, 1922, vi, 255.
(2) Benedict and Homans: Journ. Med. Research, New Series, 1912, xx, 409.
(3) Magnus Levy: Zeitschr. f. klin. Med., 1906, lx, 179.
(4) Means: Journ. Med. Research, 1915, xxxii, 121.
(5) Boothby: Journ. Amer. Med. Assoc., 1921, lxxvii, 252.
(6) McKinlay: Arch. Int. Med., 1921, xxviii, 703.
(7) Loewy and Richter: Arch. f. Physiol., Suppl., 1899, 174.
(8) Murlin and Bailey: Surg., Gynec. and Obst., 1917, xxv, 332.
(9) Luethje: Arch. f. exper. Path. u. Pharm., 1902, xlviii, 184.
(10) Zuntz: Deutsch. Zeitschr. f. Chir., 1908, xcv, 250.
(11) Korentchewsky: Zeitschr. f. exper. Path. u. Therap., 1914, xvi, 68.
(12) Rovinsky: Dissertation published in Petrograd in 1913.
(13) Danoff: Biochem. Zeitschr., 1919, xciii, 44.
(14) Korentchewsky: Russkiv Vrach, 1910, ix, 1441. (Russian).
(15) Kojima: Quart. Journ. Exper. Physiol., 1917, xi, 351.
(16) Ruchti: Biochem. Zeitschr., 1920, cv, 1.
(17) Gradinescu: Arch. f. d. gesammt. Physiol., 1913, clii, 187.
(18) Marine and Baumann: This Journal, 1921, lvii, 135.
(19) Marine and Baumann: This Journal 1922, lix, 353.
(20) Marshall and Davis: Jour. Pharm. Exper. Therap., 1916, viii, 525.
(21) Crowe and Wislocki: Johns Hopkins Hosp. Bull., 1914, xxv, 287.
(22) Aub: This Journal 1920, liv, 388.
(23) Elliott: Journ. Physiol., 1914, xlix, 38.
(24) Voit: Zeitschr. f. Biol., 1901, xli, 113.
(25) Hari: Biochem. Zeitschr., 1914, lxvi, 20.
(26) Stewart and Rogoff: Journ. Pharm. Exper. Therap., 1917, x, 1.
(27) Loeffler: Zeitschr. f. klin. Med., 1919, lxxxvii, 280.
(28) DuBois and DuBois: Arch. Int. Med., 1916, xvii, 872.
Aub and DuBois: Arch. Int. Med., 1917, xix, 823.
(29) Muirhead: Journ. Amer. Med. Assoc., 1921, lxxvi, 652.
(30) Means: Personal communication.
(31) Marine and Lenhart: This Journal, 1920, liv, 248.
(32) Aub, Bright and Uridil: This Journal, 1922, lxi, 300.
(33) Aub, Bright and Forman: This Journal, 1922, lxi, 349.
(34) Lusk: Arch. Int. Med., 1914, xiii, 673.
(35) Martin and Armitstead: This Journal, 1922, lix, 37.
(36) Evans and Ogawa: Journ. Physiol., 1914, xlvii, 446.
(37) Langlois and Garrelon: Mem. Soc. Biol., 1910, lxviii, 356.
Tomkins, Sturgis and Wearn: Arch. Int. Med., 1919, xxiv, 269.
Boothby and Sandiford: This Journal, 1920, li, 200.
(38) Plummer and Boothby: This Journal, 1921, lv, 295.

THE METABOLIC EFFECT OF ADRENALECTOMY UPON THE URETHANIZED CAT[1]

JOSEPH C. AUB, ELIZABETH M. BRIGHT AND JONATHAN FORMAN

From the Laboratories of Physiology of the Harvard Medical School

Received for publication May 9, 1922

There is evidence that the course of events following adrenalectomy progresses more rapidly in anesthetized than in unanesthetized animals. Bazett (1) has shown that urethane anesthesia causes death from adrenalectomy to occur in ten and one-half hours, whereas when the adrenals are excised under ether anesthesia, and the animals allowed to recover from the anesthetic, death does not occur so promptly. There is likewise some evidence that the adrenals are more active than normal during anesthesia. Elliott (2) in 1912 showed that when animals were anesthetized by urethane and other anesthetics their adrenals were exhausted of their normal adrenin content. Underhill (3) found that urethanized rabbits developed glycosuria after adrenalin injections more easily than did unanesthetized animals. This is probably explained by the fact that both Sollmann (4) and Aub and Wu (5) have reported that there is high blood sugar in urethane anesthesia, so that any hyperglycemia caused by adrenin injection would more readily pass the kidney threshold. The basal metabolism also seems to be higher under urethane anesthesia than prior to its administration. This may be seen by looking at the charts in paper I where it is readily seen that the giving of urethane usually raised the metabolism. This rise is of the same order of magnitude as is the subsequent fall produced by adrenalectomy as shown later in the present paper. In ten experiments, in spite of the fact that the temperature was somewhat reduced, the average metabolism under urethane is 13.2 per cent higher than before anesthesia. Table 1 in this same paper does not show this change because the average temperature of the anesthetized cats is 2°C. below the normal, and this apparently compensates for the increased metabolic rate. In cat 115, however, (paper II) the metabolic rate fell 13 per

[1] Reported at the annual Meeting of the Association for the Study of Internal Secretions, Boston, June, 1921.

cent after urethane anesthesia. The adrenal glands had been removed five days before. The total metabolic rate of the unanesthetized animal was 1.51 calories per kilo per hour. The body temperature was not constant, however, and fell from 38.5°C. before to 36.0°C. after the observation. The cat was then promptly anesthetized with urethane, and although the temperature of 37.1°C. was approximately the average found before anesthetization, the cat's metabolism had fallen 13 per cent,—to 1.32 calories per kilo per hour. Although a single observation, this is certainly suggestive of a relationship between the increased metabolism, which has usually been found under urethane anesthesia, and the adrenal glands.[2]

All of this work suggests that adrenin is secreted more rapidly under urethane than in the animal with no anesthesia. Because of this evidence, as well as Bazett's observation on the shortened duration of life, it seemed possible that the effects of adrenalectomy upon the metabolism of the cat might be more readily observed in a urethanized animal than in the unanesthetized animal, where they appear only at the onset of the symptoms due to adrenal insufficiency. A series of experiments on urethanized animals is, therefore, here reported.

Methods. The experimental procedure was similar to that described by Aub (7) in the study of basal metabolism in traumatic shock. The respiratory air was collected in small 8-liter copper spirometers, and was obtained by means of a T-shaped tracheal cannula fitted with Tissot valves. The gas analyses were made in the Haldane apparatus always in duplicate, usually in triplicate, and the usual check of room air was frequently made. The animals were prepared as follows: Urethane was given by mouth and the animal allowed to rest on a heating pad until thoroughly anesthetized. Blood pressure was taken from the carotid artery. The tracheal cannula was inserted so that the dead space after tracheotomy was about the same as normal. Intravenous injections were made by a cannula inserted in one of the femoral veins. Adrenalectomy was performed always by the lumbar route. A small incision was made about one inch from the spinal column just under the last rib, the muscles were separated and the retroperitoneal cavity entered. The adrenals are usually very easily found and removed

[2] It is interesting to point out here the elevated metabolism found in the 13 decerebrate animals (table I, paper I) in spite of their low body temperature. Cannon and Rapport (6) have recently noted evidence of a marked outpouring of adrenin in decerebrate animals,—using the denervated heart method,—and this may well explain the raised metabolism we have observed.

without the loss of any blood by this method and without entering the peritoneal cavity. In this way other organs are not disturbed, and the operation is attended with practically no shock. The temperature of the animal is kept constant by means of an electric heating pad. No experiments were run when the blood pressure fell to 80 mm. Hg. or below because of the reduced metabolism which is often present under those conditions (7). When adrenalin was injected intravenously it was usually at a dilution of 1 to 100,000 and was injected at a constant rate. At the end of the experiment a careful autopsy was made, particular attention being paid to the site of operation; and a very thorough search was made for accessory adrenal tissue in order to ascertain that adrenalectomy was complete. In none of these experiments was an accessory adrenal found.

General considerations. The accompanying tables (1, 2, 3 and 4) show that there is a prompt fall in the metabolic rate which follows the removal of the adrenal glands. This drop was usually clear cut and decisive, and usually became more marked as the time after operation increased. This is shown in table 5. In three experiments out of nineteen, however, the metabolism did not fall as much as 10 per cent, and in one there was actually a slight rise of metabolism after adrenalectomy which cannot be explained (exper. XLI). The general average of 63 periods shows an average drop of 12.0 per cent. This fall in metabolism is not due merely to the removal of tissue. Verzar (8) showed that removal of the whole pancreas in curarized animals caused a prompt fall of only 8 per cent in the metabolism. He found the weight of the pancreas to be 0.3 per cent of the total body weight. He also found (9) that removal of the spleen caused a fall of only 0.7 per cent in the oxygen absorption although it was 0.27 per cent of the total body weight. And Tangl (10) removed the kidneys—which he found were 0.7 per cent of the body weight—and obtained a drop of 8.7 per cent in the oxygen absorption. Each of these tissues is more than twenty times the weight of the adrenal glands. Elliott and Tuckett (11) found the adrenal glands of nine cats weighed 0.013 per cent of the body weight. We have found the same average figure in twenty-three cats in which the adrenals were hardened in formaline before final dissection, and the surface dried before weighing. It is clear, therefore, that the fall in metabolism cannot be accounted for merely by the amount of tissue removed, for if this were the only factor a proportionate drop in metabolism after adrenalectomy would be less than 0.5 per cent. These figures, therefore, point to the conclusion that under urethane anesthesia adrenalectomy is

TABLE 1

Effects of adrenalectomy upon the respiratory metabolism

EXPERIMENT NUMBER WEIGHT DATE	TIME		RESPIRATORY EXCHANGE PER MINUTE		RESPIRATORY QUOTIENT	CALCULATED CALORIES PER HOUR		RESPIRATORY VOLUME PER MINUTE	RECTAL TEMPERATURE	BLOOD PRESSURE	PULSE RATE PER MINUTE	REMARKS
	Post anesthesia	Post last operation	CO_2	O_2		Calories	Variation					
			cc.	*cc.*			*per cent*	*cc.*	°C.	*mm.*Hg.		
XXV	50′		17.2	23.8	0.72	6.67		444	36.2	110	156	Slight loss of blood during operation
	1° 10′		18.3	25.3	0.72	7.12		462	36.5	116	186	
	1° 57′											Double adrenalectomy completed
3300 gms. ♂	2° 13′	16′	15.3	19.6	0.78	5.58	−19	411	37.1	88	132	
1/24/20	2° 41′	44′	15.9	19.4	0.82	5.58	−19	493	36.3	94		
	3° 03′	1° 06′	16.2	19.4	0.81	5.57	−19	498	36.2	94	162	
	4° 07′	2° 10′	13.9	18.2	0.76	5.16	−26	469	36.2	85		
	4° 38′	2° 41′	14.2	18.3	0.78	5.20	−25	508	36.3	85	141	Autopsy showed large, dark spleen
XXXI	28′		27.3	33.8	0.81	9.69		826	38.2	100		
	48′		25.8	33.1	0.78	9.43		860	38.1	104	156	
	1° 09′		25.4	33.1	0.77	9.39		798	38.2	98	174	
	1° 50′											Double adrenalectomy completed
4400 gms. ♂	2° 00′	10′	21.0	31.4	0.67	8.79	− 8	602	38.6	108	189	
2/7/20	2° 20′	30′	21.8	31.9	0.68	8.94	− 6	595	38.6	108	180	
	2° 46′	56′	21.6	31.3	0.69	8.78	− 8	608	38.6	102		
	3° 50′	2° 00′	21.6	29.8	0.72	8.36	−12	676	38.5	104		
	4° 20′	2° 30′	21.7	30.1	0.72	8.38	−12	754	38.5	102	222	
	4° 50	3° 00′	20.3	28.0	0.73	7.86	−17	698	38.7	82	180	

	35′		28.4	37.9	0.75	10.71		920	39.2	132	192	
	57′		28.1	36.3	0.77	10.32		742	39.2	132	204	
	1° 19′		31.3	37.5	0.83	10.83		859	39.2	120		
	2° 17′											
XXXII												Double adrenalectomy completed
4400 gms. ♂	2° 28′	11′	23.6	31.7	0.74	8.94	−16	641	39.5	101		
2/11/20	2° 48′	31′	24.8	32.5	0.76	9.22	−13	630	39.3	106	192	
	3° 17′	1° 00′	25.4	31.9	0.80	9.13	−14	776	39.6	88		
	3° 52′	1° 35′	22.7	29.4	0.77	8.36	−21	637	39.3	98		
	4° 39′	2° 22′	21.1	26.7	0.79	7.62	−28	527	38.5	82		
	26′		17.9	21.6	0.81	6.20		978	37.5	110		
	46′		13.7	18.7	0.73	5.37		584	37.4	130	204	
	1° 04′		16.1	19.4	0.83	5.60		717	37.4	106		
	2° 07′											
XLVII												Double adrenalectomy completed. Temperature 38.2
2500 gms. ♀	2° 30′	23′	13.7	17.2	0.79	4.92	−13	402	37.4	90	216	
3/8/20	2° 54′	47′	15.4	18.7	0.82	5.37	− 5	482	37.6	92		
	3° 24′	1° 17′	14.4	17.5	0.82	5.04	−11	428	37.4	100		
	4° 11′	2° 04′	14.7	17.7	0.83	5.11	−10	402	37.3	94	192	Animal pregnant—6 fetuses (9 mm.) · Stomach contained large pieces of meat. Intestinal tract empty except lower rectum

TABLE 1—*Concluded*

EXPERIMENT NUMBER WEIGHT DATE	TIME		RESPIRATORY EXCHANGE PER MINUTE		RESPIRATORY QUOTIENT	CALCULATED CALORIES PER HOUR		RESPIRATORY VOLUME PER MINUTE	RECTAL TEMPERATURE	BLOOD PRESSURE	PULSE RATE PER MINUTE	REMARKS
	Post anesthesia	Post last operation	CO_2	O_2		Calories	Variation					
			cc.	*cc.*			*per cent*	*cc.*	°C.	*mm.*Hg.		
LX 3200 gms. ♂ 5/10/20	48′	30′	15.3	21.3	0.72	5.98		740	37.6	150	168	Thyroids out at beginning of experiment
	1° 10′	52′	13.5	19.2	0.71	5.37		648	37.4			
	1° 52′	1° 34′	12.6	17.6	0.72	4.94		442	37.3	120	240	
	2° 30′											Double adrenalectomy completed
	2° 44′	14′	9.5	14.8	0.64	4.15	−23	266	37.3	128	240	
	3° 08′	38′	11.2	16.3	0.69	4.57	−16	309	37.5			
	3° 34′	1° 04′	15.0	15.8	0.95	4.70	−13	375	37.3			Right adrenal cut in removing, but remaining portion removed promptly. Autopsy showed them entirely removed
	4° 04′	1° 34′	11.9	15.8	0.75	4.47	−17	428	37.9	110	240	
XLVI 2200 gms. ♀ 3/6/20	39′		14.3	18.0	0.80	5.14		332	36.4	122	114	
	1° 04′		12.1	16.2	0.74	4.58		263	36.3	120	120	
	1° 21′		12.8	16.6	0.77	4.72		270	36.5	102	114	
	2° 12′											Double adrenalectomy completed

XLVI 2200 gms. ♀ 3/6/20 cont.	2° 18′	6′	12.3	17.4	0.71	4.87	+ 1	310	36.5	102	162	
	3° 10′	58′	13.7	18.4	0.74	5.18	+ 7	382	36.4	114	144	
	3° 46′	1° 34′	12.1	16.2	0.75	4.57	− 5	331	36.5			On basis of one gas analysis
	4° 13′	2° 01′	12.2	16.1	0.75	4.56	− 5	325	36.4	136	168	Post mortem showed animal had cirrhosis of liver. Adrenals out completely
	4° 38′	2° 26′	13.1	17.2	0.76	4.88	+ 1	367	36.6	116	162	
LII 3000 gms. ♂ 3/22/20	1° 10′		16.0	19.1	0.83	5.52		381	37.2	100		
	1° 29′		15.6	19.9	0.79	5.68		387	37.5	95	108	
	1° 47′		15.9	18.9	0.84	5.47		396	37.7			
	2° 55′											Double adrenalectomy completed
	3° 06′	11′	14.2	18.5	0.77	5.24	− 6	341	37.7	83	132	
	3° 25′	30′	13.3	15.2	0.87	4.43	−20	318	37.4	86	132	
	3° 56′	1° 01′	12.4	16.9	0.73	4.75	−15	282	37.5	86	126	

TABLE 2

Effects of adrenalectomy upon the respiratory metabolism and blood flow

EXPERIMENT NUMBER WEIGHT DATE	TIME		RESPIRATORY EXCHANGE PER MINUTE		RESPIRATORY QUOTIENT	CALCULATED CALORIES PER HOUR		RESPIRATORY VOLUME PER MINUTE	RECTAL TEMPERATURE	BLOOD PRESSURE	PULSE RATE PER MINUTE	BLOOD FLOW PER MINUTE	REMARKS
	Post anesthesia	Post last operation	CO_2	O_2		Calories	Variation						
			cc.	*cc.*			*per cent*	*cc.*	*°C.*	*mm. Hg.*		*cc.*	
LIX 3300 gms. ♂ 5/6/20	1° 19′		19.0	25.7	0.74	7.24		497	37.8	160			
	2° 03′		20.9	26.6	0.79	7.58		539	38.2	110			
	2° 37′											383	25 cc. blood withdrawn and 38 cc. transfused
	2° 51′		19.4	26.7	0.73	7.48	+ 1	553	38.1	90	180		
	3° 19′												Both adrenals removed satisfactorily
	3° 52′	14′	19.3	25.4	0.76	7.19	− 3	624	38.5–38.2	120			Good experiment except for poor temperature regulation
	4° 26′	48′	15.5	20.2	0.77	5.74	−23	575	38.1–38.3	112	200	259	
LXXII 4200 gms. ♂ 6/4/20	1° 33′		23.3	29.9	0.78	8.50		1228	37.9	138			
	2° 05′		22.5	29.6	0.76	8.37		875	38.1	150			
	2° 25′											614	2:25, 20 cc. blood withdrawn 2:30, transfused 10 cc. B. P. 150. at 2:35
	4° 24′	1° 00′	23.2	29.4	0.79	8.39		748	38.5–38.0	126			3:25 adrenals removed completely. Some loss of blood due to slipping of ligature
	4° 48′	1° 24′	20.6	27.2	0.76	7.69	− 9	1059	37.9	125±		363	3:30, 20 cc. transfused, B. P. 112

LXXIII 3600 gms. ♂ 6/7/20	1° 14′		18.1	25.3	0.72	7.10		675	35.6	126	160	813	
	1° 35′		18.3	23.3	0.79	6.63		835	35.7	122			
	1° 55′		16.7	22.1	0.76	6.24		761	35.6				
	2° 55′												Double adrenalectomy completed
	3° 20′	25′	16.3	21.2	0.77	6.02	−10	656	36.1–35.5	120	204		
	4° 12′	1° 17′	15.1	19.9	0.76	5.63	−16	823	35.7	106			
	4° 40′	1° 45′	14.2	18.6	0.76	5.27	−21	740	35.6	120	210	520	Adrenals out completely. No accessory adrenal
LXXIV 3300 gms. ♂ 6/9/20	1° 42′		16.1	23.0	0.70	6.44		356	37.0	120	186	598	
	2° 07′		19.9	26.2	0.76	7.41		503	37.0	104	216		
	2° 53′												Double adrenalectomy completed
	3° 34′	41′	17.3	22.2	0.78	6.32	−9	632	37.1	90			
	4° 28′	1° 35′	14.4	18.7	0.77	5.30	−24	579	36.4–36.7	90			
	4° 54′	2° 01′	13.6	19.1	0.71	5.36	−23	644	36.9	90	204	385	
LXXV 2500 gms. ♀ 6/15/20	1°		16.3	20.9	0.78	5.94		473	38.1	102	140	267	
	1° 20′		17.7	21.3	0.83	6.12		433	38.5–38.6				
	2° 09′												Double adrenalectomy completed
	2° 18′	9′	12.9	19.1	0.69	5.35	−11	400	38.5–38.2	81	124		
	3° 05′	56′	16.5	20.9	0.79	5.97	− 1	465	38.8	84		260	Stomach filled with watery half-digested mass of food

TABLE 3

Effects of control operations and adrenalectomy upon the respiratory metabolism

EXPERIMENT NUMBER WEIGHT DATE	TIME		RESPIRATORY EXCHANGE PER MINUTE		RESPIRATORY QUOTIENT	CALCULATED CALORIES PER HOUR		RESPIRATORY VOLUME PER MINUTE	RECTAL TEMPERATURE	BLOOD PRESSURE	PULSE RATE PER MINUTE	REMARKS
	Post anesthesia	Post last operation	CO_2	O_2		Calories	Variation					
			cc.	*cc.*			*per cent*	*cc.*	*C.*	*mm.Hg*		
XXXVII 3900 gms. ♂ 2/21/20	32′		22.6	31.2	0.72	8.76		525	37.7	130	168	
	58′		19.1	27.4	0.70	7.67		430	37.7	105	168	
	1° 15′		21.7	29.4	0.74	8.29		466	37.8	114	168	
	2° 08′											Ligatures placed about both adrenals, but not tied
	2° 12′	4′	21.7	29.2	0.75	8.22		459	37.6	116	180	
	2° 38′	30′	22.3	28.4	0.79	8.09	− 2	476	37.8	114	192	Hemorrhage in removing adrenals caused experiment to be discontinued
XLIV 3100 gms. ♀ 3/3/20	47′		20.5	24.8	0.83	7.15		640	37.3	136	186	
	1° 42′		19.3	24.8	0.78	7.06		612	37.5	104		
	2° 35′											Left adrenal removed and ligature placed about right adrenal
	2° 40′	5′	19.0	24.7	0.77	7.01	− 1	523	38.0	118	240	
	3° 05′											Right adrenal removed
	3° 07′	2′	19.2	24.6	0.78	7.01	− 1	506	37.6	116	222	
	3° 29′	24′	19.5	22.7	0.86	6.60	− 7	535	37.4	118	198	
	3° 54′	49′	17.6	21.9	0.80	6.28	−12	455	37.5	126	234	
	4° 18′	1° 13′	18.0	22.4	0.80	6.43	−10	463	37.5	110	228	Autopsy. Animal was lactating. A small fragment of adrenal cortex found caught in ligature

XLIX 2800 gms. ♂ 3/15/20	17′		15.7	22.2	0.71	6.22		312	35.6			
	42′		14.8	21.8	0.68	6.10		301	35.8	168	198	
	1° 39′											Left adrenal removed
	1° 53′	14′	17.3	24.1	0.72	6.75	+10	442	35.8	120		
	2° 20′											Right adrenal removed
	2° 24′	4′	16.1	22.4	0.72	6.29	+ 7	382	35.9	124	204	
	2° 52′	32′	12.6	19.1	0.66	5.37	−13	257	35.8	125	210	
	3° 47′	1° 27′	14.1	20.3	0.70	5.70	− 8	282	35.7	126	186	Temperature rose to 37.6 before this period
XLII 2450 gms. ♂ 3/1/20	55′		17.5	21.7	0.81	6.23		371	37.7	150	171	Ligatures in place about adrenals before respiratory observations. Otherwise no manipulation
	1° 20′		19.0	22.2	0.86	6.45		444	37.6	124	177	
	1° 32′		18.8	21.8	0.87	6.34		498	37.7	114	168	
	2° 18′		17.9	21.4	0.83	6.19		453	37.5	102	177	
	2° 38′		19.7	22.0	0.89	6.44		581	37.4	112	201	

TABLE 4

Effects of adrenalectomy and adrenalin injections upon the respiratory metabolism

EXPERIMENT NUMBER WEIGHT DATE	TIME		RESPIRATORY EXCHANGE PER MINUTE		RESPIRATORY QUOTIENT	CALCULATED CALORIES PER HOUR		RESPIRATORY VOLUME PER MINUTE	RECTAL TEMPERATURE	BLOOD PRESSURE	PULSE RATE PER MINUTE	REMARKS
	Post anesthesia	Post last operation	CO_2	O_2		Calories	Variation					
			cc.	*cc.*			*per cent*	*cc.*	*°C.*	*mm.*Hg.		
XXXVI 2600 gms. ♂ 2/19/20	29′		16.0	20.4	0.79	5.80		432	37.6	122		
	47′		14.4	19.3	0.75	5.43		376	37.6	128	198	
	1° 05′		16.0	20.0	0.80	5.73		406	37.7	130	210	
	1° 45′											Manipulation, ligatures in place but not tied
	1° 50′	5′	13.4	19.4	0.69	5.43	− 4	313	37.6	104	192	
	2° 12′	27′	14.3	19.0	0.75	5.37	− 5	348	37.6	96		
	2° 40′											Double adrenalectomy completed ligatures in place
	2° 47′	7′	12.2	17.3	0.70	4.85	−14	285	37.8	82		
	3° 16′	36′	11.2	16.6	0.68	4.63	−18	260	37.6	94	198	
	3° 50′											Began injection of adrenalin chloride, 0.004 mgm. per kilo per minute. Continued for 24 minutes
	3° 55′	1° 15′	16.3	17.3	0.94	5.13	− 9	386	37.2	104–80		
	4° 15′	1° 35′	16.8	19.6	0.86	5.69	+ 1	453	37.5	110	228	
	5° 47′	3° 07′	14.7	20.0	0.74	5.63	− 1	436	37.6	94		

XLI 3900 gms. ♂ 2/27/20	1° 26′		21.0	26.9	0.78	7.65		817	37.5	126	168	
	1° 58′		17.7	24.0	0.76	6.76		693	37.8	98	168	
	2° 44′											Manipulation of adrenals complete. Ligatures in place.
	2° 54′	10′	20.2	25.0	0.81	7.16	− 1	659	36.9	104		
	3° 14′	30′	23.7	27.4	0.86	7.98	+10	784	37.6	112	156	
	3° 55′											Double adrenalectomy completed
	4° 04′	9′	17.2	24.9	0.69	6.98	− 3	457	38.0	138	156	
	4° 30′	35′	22.1	28.3	0.78	8.06	+11	570	38.1	138	168	
	5° 04′											Injection adrenalin chloride, 0.005 mgm. per kilo for 10 minutes
	5° 11′	1° 09′	30.6	30.6	1.00	9.13	+21	786	37.9	110	162	
			25.5	31.5	0.81	9.03	+20	663	38.3	135	174	An unusual result. Shows striking effect of continuous adrenalin injections
LVII 2900 gms. ♂ 4/6/20	57′	48′	14.8	20.5	0.72	5.74		389	36.7			Thyroids out at beginning of experiment
	1° 34′	1° 25′	15.8	21.7	0.73	6.11		405	37.3	116		
	2° 15′											Double adrenalectomy complete
	2° 22′	7′	11.8	19.1	0.62	5.36	− 9	300	36.6	116	159	
	2° 44′	29′	12.6	19.0	0.66	5.33	−10	313	36.5	124		
	3° 44′	1° 29′	11.3	16.6	0.68	4.66	−21	283	37.0			Temperature 38.0 half-hour preceding this sample
	4° 45′	2° 30′	11.7	16.7	0.70	4.68	−21	287	36.5			
	5° 30′											Adrenalin injected for 11 minutes, 0.004 mgm. per kilo per minute
	5° 41′	3° 23′	15.9	19.6	0.81	5.63	− 5	468	35.7	120		

TABLE 4—*Continued*

EXPERIMENT NUMBER WEIGHT DATE	TIME		RESPIRATORY EXCHANGE PER MINUTE		RESPIRATORY QUOTIENT	CALCULATED CALORIES PER HOUR		RESPIRATORY VOLUME PER MINUTE	RECTAL TEMPERATURE	BLOOD PRESSURE	PULSE RATE PER MINUTE	REMARKS
	Post anesthesia	Post last operation	CO_2	O_2		Calories	Variation					
			cc.	*cc.*			*per cent*	*cc.*	*°C.*	*mm.Hg.*		
LV 2900 gms. ♂ 4/2/20	28′	23′	16.5	27.3	0.60	7.32		475	37.5	160		Thyroids out at beginning of experiment
	48′	43′	20.6	29.5	0.70	8.27		607	37.4			
	1° 05′	1° 00′	20.2	29.7	0.68	8.31		636	37.5			
	2° 07′											Double adrenalectomy completed
	2° 20′	13′	17.5	25.5	0.69	7.16	−10	496	37.7	120		
	3° 01′	54′	16.4	23.1	0.71	6.48	−19	500	37.6			
	3°-59′	1° 52′	14.7	21.6	0.68	6.04	−24	401	37.5			
	4° 32′	2° 25′	16.7	23.8	0.70	6.66	−17	460	37.2	116		
	5° 05′											Adrenalin injected for 10 minutes, 0.004 mgm. per kilo per minute
	5° 34′	3° 27′	15.7	23.1	0.68	6.48	−19	560	37.5			
CCXII 3810 gms. 12/15/21	1° 15′		26.1	30.5	0.86	8.84		1124	37.5	92		
	2° 25′		25.9	29.2	0.89	8.53		865	37.5			
	2° 40′											Perfused with 20 cc. fresh blood
	2° 42′		25.1	31.7	0.79	9.07		980	37.4	100		
	3° 00′											Adrenalin injected for 15 minutes, 0.004 mgm. per kilo per minute

CCXII 3810 gms. 12/15/21 cont.	3° 06′		29.9	32.7	0.92	9.61	+13	1067	37.5			Sciatic and brachial plexuses cut. Perfused with 15 cc. blood
	3° 25′		24.0	30.7	0.78	8.75	− 1	914	37.6	100		
	4° 20′		20.2	26.5	0.76	7.52	−15	599	37.4	79		
	4° 45′		23.8	30.4	0.79	8.66	− 2	795	37.2	79		
LXXVI 2600 gms. 2/23/21	34′		17.5	22.9	0.76	6.48		1386	37.4	146		
	58′		18.0	23.9	0.75	6.75		1404	37.7			
	1° 30′											Injected mammalian Ringer's solution, 1 cc. per minute
	1° 30′		15.4	21.2	0.72	5.95	−10	801	37.9			
	2° 09′											Adrenalin injected for 10 minutes, 0.004 mgm. per kilo per minute
	2° 10′		18.9	22.0	0.86	6.38	− 4	681	38.1	134		
	2° 28′		12.6	18.5	0.68	5.19	−22	435	37.7	154		
	2° 57′		13.6	19.7	0.69	5.52	−17	373	37.4	166		
	3° 28′											Adrenalin injected for 17 minutes, 0.004 mgm. per kilo per minute
	3° 40′		14.8	20.3	0.73	5.72	−14	458	38.2	148		
	4° 06′		17.5	23.3	0.75	6.60	±0	547	38.2	186	156	
LXXVII 2100 gms. 6/15/21	30′		16.8	24.6	0.68	6.90		711	36.4	110		
	1° 10′		16.2	19.9	0.81	5.72		699	36.2	94	168	
	1° 50′											Double adrenalectomy completed
	2° 00′	10′	17.1	21.7	0.79	6.20	− 2	605	36.7	98	198	
	2° 30′	40′	16.5	21.0	0.78	5.99	− 5	639	36.8	92		
			16.7	20.5	0.82	5.89	− 7	739	36.6	92	207	

TABLE 4—*Concluded*

EXPERIMENT NUMBER WEIGHT DATE	TIME		RESPIRATORY EXCHANGE PER MINUTE		RESPIRATORY QUOTIENT	CALCULATED CALORIES PER HOUR		RESPIRATORY VOLUME PER MINUTE	RECTAL TEMPERATURE	BLOOD PRESSURE	PULSE RATE PER MINUTE	REMARKS
	Post anesthesia	Post last operation	CO_2	O_2		Calories	Variation					
			cc.	*cc.*			*per cent*	*cc.*	*C.*	*mm.Hg.*		
LXXVII	3° 20′	1° 30′										Adrenalin injected for 19 minutes, 0.004 mgm. per kilo per minute
2100 gms.												
6/15/21	3° 26′	1° 36′	18.3	23.0	0.80	6.57	+ 4	744	36.5	92		
cont.	3° 42′	1° 51	17.9	24.5	0.73	6.88	+ 8	729	36.7	116	201	
	4° 08′	2° 18′	15.2	20.9	0.73	5.86	− 7	533	36.2			
		54′	34′	19.5	26.7	0.73	7.50		485	37.7	118	144	Thyroids out at beginning of experiment
	1° 20′	1° 00	22.0	28.3	0.78	8.05		520	37.6			
	1° 30′	1° 10′	21.5	28.1	0.77	7.98		534	37.9	114	174	
	2° 25′											Double adrenalectomy completed
LIII												
5100 gms. ♂	2° 49′	24′	17.2	25.1	0.72	7.03	−10	432	37.8	132	174	
3/25/20	3° 40′	1° 15′	19.5	26.8	0.73	7.52	− 4	483	37.4	122	174	
Castrated	4° 13′	1° 48′	19.4	27.0	0.72	7.57	− 3	494	37.8	146	168	
	4° 40′											Injection intravenously 0.2 cc. adrenalin chloride, 1:1000
	4° 44′	2° 19′	23.1	25.7	0.90	7.54	− 4	600	37.7	60–120		Calculated on basis of one analysis only

followed by a prompt reduction of the metabolic rate of far greater magnitude than the mere removal of tissue would warrant.

It is likewise clear that the operation we performed produces no more trauma or shock than does removal of the pancreas or kidney, so that this cannot account for the metabolic effect. This is more clearly demonstrated in our control experiments. In these the operation incisions were completed, the adrenals exposed, all the ligatures put in place but not tied, so that the blood supply remained nearly intact. This procedure produced no average effect on the metabolic rate in eight periods (table 3 and expers. XXXVI and XLI, table 4). When now the ligatures were tied and the adrenals easily removed, the usual fall in metabolism appeared.

TABLE 5

Effect of adrenalectomy upon the metabolic rate in cats under urethane anesthesia

TIME AFTER ADRENALECTOMY	NUMBER OF OBSERVATIONS	PERCENTAGE VARIATION FROM NORMAL FOUND BEFORE OPERATION
minutes		
0– 30	17	– 9.2
30– 60	17	– 9.1
60– 90	11	–12.7
90–120	7	–16.6
120–150	7	–16.9
150–180	3	–19.1
180–210	1	–17.3
Average.....	9	–12.0

There is the usual fall in metabolism after adrenalectomy even though the thyroid glands are first removed as is demonstrated by experiment LX (table 1) and experiments LV and LVII (table 4). This is evidence of the independence of the effects of these two glands, as it shows that the fall in metabolism following adrenalectomy, in these experiments at least, is not due to an indirect action upon the thyroid. This is interesting in regard to the recent suggestion of Marine and Baumann (12) of the interrelationship of the thyroid and adrenal. Such an explanation has also been suggested by Korentchewski (13) for the reduced metabolism which develops several weeks after castration, but does not appear so strikingly if the thyroids are first removed. It is nearly a month before this change becomes distinct, however, and therefore the change in the metabolism which we have found in these

acute experiments after adrenalectomy is a different type of reaction, because of the rapidity of onset.

The question, of course, arises as to whether this fall in metabolism following adrenalectomy is due to a markedly decreased blood flow,—such as accompanies a similar fall in traumatic shock (14). The five experiments in this series (table 3) were studied by the same methods as were used in the study of shocked animals.[3] While there is a fall in the blood flow per minute after adrenalectomy, it is not much greater than that shown by the control experiments which have been previously reported (14). The blood for the determination was taken immediately after the collection of the respiratory gases. Recent work by Nakamura (15) shows that in muscle, at least, blood flow changes of this magnitude could not cause a fall of metabolic rate. Changes in the work of the heart or of respiration might possibly be a cause for the fall in metabolism, for these are the most evident activities of an anesthetized animal. Such a cause for the change is ruled out, however, by experiments LXXIII and LIX (table 2) and by experiment XXXI (table 1) in which the volume of respiration and the blood pressure remain about constant and the pulse increases in rate. In these three experiments, therefore one should expect a rise rather than a fall of metabolism if the cardio-respiratory work were the cause of the metabolic changes.

Whether this fall in the rate of metabolism was due to lack of adrenal cortex or medulla was next to be investigated. In two experiments adrenal cortex preparations were injected intravenously and the metabolism followed for several hours after the injection. One preparation[4] consisted of the adrenal cortex with lipoids extracted. The other was made by careful dissection of the adrenal cortex of oxen and then extracting the lipoids with ether. When the ether was completely evaporated these lipoids gave a negative test for arenalin by the Folin Cannon, Dennis method. Neither preparation showed any effect on the metabolic rate for three hours after injection. A similar negative result for a short period was obtained following the intravenous injection of thyroxin, which confirms experiments from the Mayo Clinic. Adrenalin, however, gave more positive results when it was given by continuous intravenous injections. These were made at approximately a "physiological" rate, so that the conditions were analogous to what

[3] All the blood gas analyses and blood flow calculations were made by Mr. F. G. Thompson, whom we take this opportunity to thank.

[4] We wish to take this opportunity to thank Parke, Davis & Co. for furnishing us with the preparation.

may normally occur. This was based on the work of Stewart and Rogoff (16) who have tested the rate of adrenal secretion in the experimental animal. When they used the eye reactions they concluded the average rate was 0.0006 mgm. per kilo per minute; when they used the intestine and uterus segments they decided the average rate of flow was 0.00025 mgm. per kilo per minute, the lower result being ascribed by them to loss of epinephrin in the "assay" method. Cannon and Rapport (17) have found that in emergencies this rate may be ten times the above values. In the intravenous injections which we made, the rate averaged 0.004 mgm. per kilo per minute, the duration of injection was usually only ten minutes and the effects watched for prolonged periods after the injection had stopped. This series consisted of seven experiments and did not show absolutely consistent results. However, in five of the experiments there was a distinct rise of the metabolic rate following the adrenalin injection. This is best illustrated in experiment XXXVI where the metabolism fell 18 per cent below normal after removal of the adrenals and then returned to the normal value after injection of adrenalin. In one experiment (no. LIII) there is a slight rise only. In experiment LV the result was negative, but only one period was taken 15 to 25 minutes after the adrenalin injection had stopped and so possibly after the metabolic effect had disappeared. The usually striking rise following continued intravenous injection was not noted after intramuscular injections or after single intravenous injections of small quantities of adrenalin (exper. LIII, table 4). That intravenous injections of adrenalin would increase the metabolic rate has already been noted by Langlois and Garrelon (18) who gave repeated intravenous injections of 0.5 to 1.0 mgm. They kept the blood pressure raised by this method for 10 to 15 minutes and found a distinct increase of the metabolism during this period. Of course, numerous observers have noted the effects on the metabolic rate of large doses of adrenalin, (1 cc.) given subcutaneously. It is clear, therefore, that adrenalin, when injected at a quite physiological rate may cause a rise of metabolism which is analogous in magnitude to the fall produced by removing the adrenal glands.

CONCLUSIONS

1. Evidence is collected that suggests a greater flow of adrenalin under urethane anesthesia than normal.

2. After removal of the adrenal glands under urethane anesthesia, there is a prompt and progressive fall of metabolism which averages 12 per cent.

3. This drop occurs after removal of the thyroid gland. The thyroid, therefore, does not seem to be essential to this reaction.

4. Adrenal cortex extracts have no prompt effects on the metabolic rate.

5. Adrenalin injected intravenously at a physiological rate causes a distinct rise in the metabolism.

6. The conclusion is suggested that in these experiments the fall in metabolism after adrenalectomy was due to the lack of the usual adrenal secretion.

BIBLIOGRAPHY

(1) Bazett: Journ. Physiol., 1919, liii, 320.
(2) Elliott: Journ. Physiol., 1912, xliv, 374.
(3) Underhill: Journ. Biol. Chem., 1911, ix, 13.
(4) Sollmann: A manual of pharmacology, 1917.
(5) Aub and Wu: This Journal, 1920, liv, 416.
(6) Cannon and Rapport: This Journal, 1921, lviii, 337.
(7) Aub: This Journal, 1920, liv, 388.
(8) Verzar: Biochem. Zeitschr., 1912, xliv, 201.
(9) Verzar: Biochem. Zeitschr., 1913, liii, 69.
(10) Tangl: Biochem. Zeitschr., 1911, xxxiv, 1.
(11) Elliott and Tuckett: Journ. Physiol., 1906, xxxiv, 332.
(12) Marine and Baumann: This Journal, 1922, lix, 353.
(13) Korentchewski: Zeitschr. f. exper. Path. u. Therap., 1914, xvi, 68.
(14) Aub and Cunningham: This Journal, 1920, liv, 408.
(15) Nakamura: Journ. Physiol., 1921, lv, 100.
(16) Stewart and Rogoff: Journ. Pharm. Exper. Therap., 1917, x, 1 (see p. 3).
(17) Cannon and Rapport: This Journal, 1921, lviii, 308.
(18) Langlois and Garrelon: Mem. d. l. Soc. d. Biol., 1910, lxviii, 356.

PRELIMINARY DILATATION A PHASE OF THE PUPILLARY LIGHT REFLEX

JOSEPH BYRNE

From the Department of Biology, Fordham University, New York

Received for publication May 11, 1922

For some years the author has observed in animals (cats mostly) and man that immediately following sudden retinal illumination, e.g., by a pocket flashlight, the pupil dilates as a preliminary to constriction. The phenomenon is elicited indirectly (consensually) as well as by direct illumination. The present study is an attempt to elucidate the mechanism involved.

Preliminary dilatation, direct and consensual, is not abolished by removal of the superior cervical ganglion, section of the cervical sympathetic, complete transection or hemi-transection of the cervical spinal cord. It is abolished however by section of the oculo-motor nerve or the equivalent of this, viz., removal of the ciliary ganglion, section of the short ciliary nerves or lesions in man causing ophthalmoplegia. It is also abolished in conditions associated with hypertonus of the constrictor mechanism, viz., sleep, coma and accommodation (convergence). These observations were made on numerous animals (cats) normal and after experimental lesions, and in man after pathological lesions as well as in the normal waking and sleeping state. In some cases of locomotor ataxia, under observation for long periods, preliminary dilatation was found to persist as long as there was any trace of constriction to light. Stimuli such as loud noise, pin-pricking, surprise, or fright of any kind caused the pupil to dilate but under these circumstances the pupil did not rebound immediately in sudden constriction as it did when dilatation had been induced by retinal illumination. Preliminary dilatation is more readily elicited in young than in older animals. The latter frequently fix the pupils in accommodation which abolishes the phenomenon. The effect of various drugs was studied. During the administration of ether preliminary dilatation disappeared early, far in advance of the wink reflex. Coincident with the disappearance of preliminary dilatation was the complete

disappearance of constriction to light. Cocaine enhanced rather than abolished the phenomenon. Thus 40 minutes after instillation of a 2 per cent solution of cocaine hydrochloride the pupils were of equal size at about 10.0 mm. and preliminary dilatation—direct and consensual—was present to an extent greater than normal for this animal and far greater than what would be expected at that degree of dilatation. Instillations of a 1 per cent solution of homoatropine caused at first a marked reduction in the response and finally complete abolition of the phenomenon. Instillations of a 0.5 per cent solution of eserine sulphate and of a 1 per cent solution of pilocarpine hydrochloride caused at first reduction and later abolition of the phenomenon. Ergotoxine gr. $\frac{3}{5}$ given intravenously to a cat 5 pounds weight, failed to completely abolish the phenomenon but modified it in such a way that

Fig. 1 Fig. 2

Fig. 1. Flash light photograph showing preliminary dilatation.

Fig. 2. Same man in ordinary daylight just before taking the flashlight photograph.

the preliminary dilatation was of extremely short range, apparent merely as a hesitancy in constriction, during the height of the action of the drug the ensuing constriction being slow and apparently of much longer range than normal. These movements were followed by a third, viz., dilatation which was fairly brisk and easily discernible. During the period of recovery from the drug each of the three phases of pupillary movement was marked, the preliminary dilatation becoming seemingly more accentuated than the others.

The photographs show the pupillary alteration incidental to the sudden illumination in taking a flashlight photograph. The effect is of course not altogether the result of retinal illumination, although it well illustrates preliminary dilatation. The moving pictures of the pupil, following retinal illumination, taken by Weiler (1) in 1910 show

clearly the preliminary dilatation studied in the present paper although Weiler failed to appreciate it as a phenomenon apart from the latent period of the constrictor mechanism.

DISCUSSION AND SUMMARY. The facts enumerated point to preliminary dilatation as a phase of the pupillary light reflex and to reflex constrictor inhibition as the main factor in its mechanism. Evidently the efferent dilator paths that lie in the cervical sympathetic, and their continuation in the long ciliary nerves, are not an essential part of the mechanism. Some dilator tonus is however necessary for the exhibition of the phenomenon, such tonus being supplied after removal of the superior cervical ganglion, and even after full doses of ergotoxine, by local neuro-muscular dilator mechanisms. Compare the mechanism of paradoxical pupil dilatation and of intestinal movements after isolation from the cerebro-spinal system. The mechanism of preliminary dilatation is presumably identical in its main feature, viz., constrictor inhibition, with that of dilatation following sudden noise, injurious or potentially injurious stimulation, or surprise of any kind. In these latter instances the afferent paths belong to the noci-ceptive system, the reaction in pupil dilatation being contributory to the general reaction to noxious stimulation actual or potential and imminent. In the case of preliminary dilatation to retinal illumination the stimulus also acts presumably through the noci-ceptive system. This implies that in the optic nerve, as elsewhere in the general sensory nerves, are to be found functioning side by side representatives of the two fundamental afferent systems, viz., the noci-ceptive (affective) which function throughout the body in a coöperation of antagonism in integrating the mechanisms of instinctive and cognitive adjustments. Compare the work of Waller (2) who found in the excised eye of the frog "that the retina when exposed to light is the seat of two contrary electro-motive changes." Compare further the findings of Kühne and Steiner (3) which are in substantial agreement with those of Waller. Care must be taken, however, in interpreting the significance of such galvanometric experiments as it is well known that in almost all forms of nerve stimulation double phase of opposite effect is detectable. As the phenomenon is abolished in man by lesions in the nucleus, as well as in the course of the third cranial nerve, and as it may be readily elicited consensually in both man and animals (above the rabbit) the site of constrictor inhibition in preliminary dilatation is presumably the constrictor center in the oculo-motor nucleus. The early disappearance of the phenomenon in ether anesthesia would involve a difficulty were

it not for the fact that the light constriction (active) reflex seems in reality to be abolished before preliminary dilatation. The constriction observed in association with preliminary dilatation just before the abolition of the latter is predominantly a passive movement executed in obedience to constrictor tonicity mainly of local origin. Compare the miosis of sleep and coma. The absence or diminution of the phenomenon in cerebral anoxemia, during accommodation and following the local application of drugs which act locally in causing constrictor hypertonus, supports the view that the mechanism of preliminary dilatation consists mainly of constrictor inhibition. The slow short-lived constriction observed after ergotoxine was the result of feeble accommodation in a partially asphyxiated animal in which the constrictor center had manifestly become anoxemic. A similar phenomenon was observed in the asphyxia incidental to a large dose of pilocarpine hydrochloride given subcutaneously. The shortness of the physiological lost time indicates that the phenomenon is a true reflex and not merely a secondary incident in a general reaction.

CONCLUSIONS

1. Preliminary dilatation is a phase of the pupillary light reflex. It is a true reflex and not merely the sequel of a general reaction.

2. The mechanism consists of constrictor inhibition taking place at the constrictor center in the oculo-motor nucleus through the medium of centripetal impulses passing over the optic nerve.

3. The optic nerve like the general sensory nerves contains representatives of the two fundamental afferent systems, viz., the nociceptive (affective) and the proprio-ceptive (critical) which function in a "coöperation of antagonism" in integrating the mechanisms of instinctive and cognitive adjustment.

4. The dilator element in preliminary dilatation in reality survives the light constrictor element in ether anesthesia and allied conditions just as it does in all conditions in which both elements are simultaneously and evenly affected by the same toxic influence.

BIBLIOGRAPHY

(1) WEILER: Zeitschr. f. d. gesammt. Neurol. u. Psychiat., 1910, ii, 1150.
(2) WALLER: The signs of life, London, 1903.
(3) KUHNE AND STEINER: Physiol. Untersuch. Heidelberg, 1880, iii, 327; 1881, iv, 564.

CALCIUM PHOSPHATE METABOLISM IN THE DIAGNOSIS OF RICKETS

J. F. McCLENDON

From the Laboratory of Physiological Chemistry, University of Minnesota

Received for publication May 13, 1922

The study of rickets has taken on a new aspect since the discovery of vitamines. According to Parke, McCollum and their co-workers, there are two kinds of rickets,—one due to lack of calcium and one due to deficiency in phosphoric acid in the diet, and cod liver oil benefits these conditions; in my experiments, however, low Ca has led to osteoporosis and not rickets. McCollum and his co-workers (1) found that butter fat was not nearly so effective as cod liver oil in the prevention of rickets, and in fact butter fat had only very slight action.

If rickets in rats is very severe the ribs are so soft that they cannot hold out the chest wall and it sinks in, thus decreasing the vital capacity. Many of these rats die and I think it is proper to say that some of these rats die as the result of rickets. Just what vital capacity is necessary to keep them alive has not been ascertained, but certainly they need more vital capacity at certain times than others, especially when inflammatory processes due to infection tend to reduce it. It seems probable that these rats die due to the fact that what we might call the vital-capacity-reserve was so reduced that they could not stand the slight bronchitis affecting all the rats. Perhaps the lowered vital capacity may lessen the oxygenation of the brain.

Experimental. By separating an animal roughly into skeleton and non-skeleton and ashing the parts separately, I found that in a white rat of about 70 grams in weight the skeleton contains 96 per cent of the calcium and 82 per cent of the phosphorus of the body. The phosphorus to calcium ratio is 0.5 in the skeleton and 0.6 in the body. On the basis of two such rats the P content was about 5 mgm. per gram body weight. Just after birth we would expect the rats to store phosphorus in order to form the phosphatides of the brain, and this process might be still continuing in rats of 30 grams but would not be noticeable in rats of 100 grams. I therefore decided to determine the calcium phos-

phate retention in normal rats of these two ages. In the following table five rats one month old of litter 20 were placed in metabolism cages where they remained one week and the average calcium and phosphorus retention per rat per day determined. When they were 57 days old

TABLE 1

Experiment I. Litter 20

RAT NUMBER	SEX	WEIGHT ON 31ST DAY	WEIGHT ON 38TH DAY	GAIN PER DAY	RETENTION PER DAY Ca	RETENTION PER DAY P	P/Ca	RACHITIC INDEX
		grams	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*	
I	♀	22.5	32.5	1.4	10.3	6.6	0.582	−1.5
II	♀	26.5	38.0	1.6	17.5	10.0	0.512	−4.5
III	♂	26.0	38.0	1.7	13.8	8.8	0.637	−3.0
IV	♂	21.5	31.5	1.4	12.5	6.2	0.5	−1.1
V	♂	30.0	44.1	2.1	17.0	8.5	0.5	−1.9
Average.....		25.1	36.9	1.64	14.2	8.2	0.567	
Grand average..... 30.9								
Retention per gram body weight per day ...					0.46	0.265		

TABLE 2

Experiment II. Litter 20

RAT NUMBER	WEIGHT ON 57TH DAY	WEIGHT ON 60TH DAY	GAIN PER DAY	FOOD INTAKE PER DAY	RETENTION OF Ca PER DAY	RETENTION OF P PER DAY	P/Ca	RACHITIC INDEX
	grams	*grams*	*grams*		*mgm.*	*mgm.*	*mgm.*	
I	76.0	79.0	1.0	8.31	19.7	9.15	0.46	−5.0
II	82.5	89.5	2.3	9.56	31.2	11.3	0.36	−4.2
III	101.5	110.5	3.0	10.38	34.0	11.1	0.33	−2.6
IV	96.5	108.0	3.8	13.15	36.6	14.3	0.37	−3.7
Aver-age..	89.1	96.8	2.5		30.4	11.4	0.375	
Grand average.....92.95								
Retention per gram body weight per day...					0.33	0.12		

they were again placed in metabolism cages and remained for three days and their calcium and phosphorus metabolism determined. Diet 94 was used the whole life of these rats and also had been fed to the mother. The composition of this diet was: whole wheat flour 28 per cent, rolled oats, 28 per cent, corn meal, 28 per cent, flax seed meal 9.5 per cent,

sodium chloride 0.5 per cent, bone meal 1.5 per cent, whole milk powder 4.5 per cent, Ca by analysis 0.79 per cent, P by analysis 0.57 per cent.

If the three-day period in the second experiment is sufficient for definite conclusions we find the P/Ca ratio lower than in the skeleton. Evidently the skeleton is changing in composition and perhaps a relatively large part of its calcium carbonate is deposited during the second experiment. Rats can be maintained at constant weight without rickets, and McCollum (2) shows that starvation will cure rickets. Normal animals when fed so little as to just maintain body weight (3) retain calcium phosphate, and their bones grow. In order to maintain a growing rat at constant weight the integument must shrink while the hard parts grow a little (4).

If we could determine the growth of the skeleton during life and compare it with the calcium phosphate retention, the diagnosis of rickets would be easy without killing the animal. Owing, however, to unequal growth of skeleton and hard parts and changes in water content of the body, an approximate empirical formula was necessary for the diagnosis and this I call the rachitic index. To find the rachitic index, determine the average daily increase in the body weight during the metabolism period and multiply it by 2.2 and subtract from this product the "corrected" daily P retention (= daily P retention minus 2). The object of subtracting 2 from daily P retention is that in the absence of growth in body weight the skeleton may grow in one day enough to require 2 mgm. P. If the rachitic index is negative the animal is normal (or it may be osteoporotic). If it is positive the animal has rickets. If the metabolism period is sufficiently long the magnitude of the rachitic index *should* be proportional to the degree of rickets. Dr. C. M. Jackson will report on the morphologic examination of these same rats.

The first experiment was made to determine whether the phosphoric acid from different sources was equally effective or ineffective in the prevention of rickets. The following diets were made up and ten rats of litter 12 were fed on the following diets.

From the analysis of the diets it would seem that diets 57 to 61, which contained approximately 0.9 per cent calcium and 0.13 per cent phosphorus, showed the same degree of rickets under the x-ray and about the same calcium retention and phosphorus deficiency, and therefore we may conclude that whether 23 per cent of the phosphorus is derived from casein, spinach, yeast or wheat germ, it is ineffective in the prevention of rickets unless the total P is increased. Rat III receiving yeast lost weight and hence had a lower rachitic index. Diets 62 to 66

consisted of diets 57 to 61 to each of which the same amount of potassium-acid-phosphate had been added in order to bring up the phosphorus

TABLE 3

RAT	DIET	FLOUR	CASEIN	SPINACH	NaCl	"LIME"		Ca	P
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		*per cent*	*per cent*
I	57	85	10	1	2	2	Yeast	0.931	0.148
III	59	93	0	1	2	2	2	0.894	0.138
IV	60	90	0	6	2	2	Wheat germ	0.971	0.125
V	61	91	2	1	2	2	2	0.905	0.132

Diet 58 = diet 57 + 100 mgm. cod liver oil per rat per day.

Diets 62–66 = diets 57–61 with 0.2 per cent flour substituted by KH_2PO_4.

Diet 62 = 0.962 per cent Ca and 0.222 per cent P, diet 64 = 0.900 per cent Ca and 0.171 per cent P.

Diet 65 = 0.970 per cent Ca and 0.173 per cent P, diet 66 = 0.923 per cent Ca and 0.181 per cent P.

Diet 63 = diet 62 + 100 mgm. cod liver oil per rat per day.

TABLE 4

Litter 12

1 month old when placed on diets

RAT	DIET	BODY WEIGHT		MILLIGRAMS PER RAT PER DAY		X-RAY	RACHITIC INDEX
		45th day	52nd day	P balance	Ca balance		
		grams	*grams*				
I	57	35.0	38.0	−1	+2	Rickets	4.0
III	59	34.0	29.0	−1	+1	Rickets	1.3
IV	60	41.0	45.0	−2	+1	Rickets	5.0
V	61	33.5	35.0	−1	+4	Rickets	3.5
II	58	28.0	28.0	−3*	+2	(Died early)	5.0
		54th day	61st day				
VI	62	44.0	50.5	+2	+2	Rickets	2.0
VIII	64	45.0	47.5	+1	+5	Rickets	1.8
IX	65	52.0	57.5	+1	+4	Rickets	2.9
X	66	38.0	41.0	+1	+4	Rickets	2.0
VII	63	46.5	47.5	+5	+12	No rickets	−2.7

*Pneumonia.

content to about 0.175 per cent; the calcium content remaining the same. This caused a positive balance of calcium and the rachitic index was lowered on the average, but not in all individual cases. The addi-

tion of cod liver oil to diet 63 without changing the mineral content trebled the calcium phosphate retention and prevented rickets. The lime used was air-slacked lime (chiefly $CaCO_3$) containing a little calcium hydrate (and probably MgO) and 0.05 per cent P. Its decomposition in the stomach would change the phosphates to basic phosphates and I observed previously that on feeding basic phosphates they seem largely to pass through the alimentary canal unchanged, judging by their shadows in the x-ray. It was therefore decided to run an experiment in which the acid balance of the diet varied very greatly. In this experiment ten rats of litter 15 were fed on the diets 72 to 83.

TABLE 5

RAT	DIET	ANALYSIS		WEIGHT OF RAT	
		P	Ca	1st day	5th day
		per cent	*per cent*	*gms.*	*gms.*
I	72 consisted of A + 0.22 per cent NaH_2PO_4	0.1762	0.820	24	23
II	73 consisted of A + 0.11 per cent NaH_2PO_4	0.165	0.859	29	27
III	74 consisted of A + 0.29 per cent Na_2HPO_4	0.1606	0.857	19	18
IV	75 consisted of A + 0.3 per cent Na_3PO_4	0.160	0.820	21	20
V	76 consisted of A + 0.3 per cent Na_3PO_4 + 0.5 per cent MgO			19	(18)
VI	77 consisted of B + 0.11 per cent NaH_2PO_4	0.167	0.583	25	30
VII	78 consisted of B + 0.11 per cent NaH_2PO_4 + 5.0 per cent $MgSO_4$	0.160	0.583	19	23
VIII	79 consisted of B + 0.22 per cent NaH_2PO_4	0.1926	0.591	30	35
IX	80 consisted of B + 0.33 per cent NaH_2PO_4	0.200	0.591	24	26
X	81 consisted of B + 0.43 per cent NaH_2PO_4	0.235	0.552	34	36

These diets were made from two stock mixtures A and B. Mixture A contained NaCl 2 per cent, lime 1.8 per cent, casein 10 per cent, spinach 1 per cent, yeast 0.1 per cent, flour 85.1 per cent. Mixture B contained NaCl 2 per cent, plaster of Paris 2 per cent, casein 10 per cent, spinach 1 per cent, yeast 0.1 per cent, flour 84.9 per cent.

All the rats fed lime started to decrease in weight and all those fed plaster of Paris to increase in weight. The difference might seem mainly to be the acid base balance, the acid in the lime being CO_2 and passing out as the food reached the stomach, but in experiments in which 5 per cent to 10 per cent sodium bicarbonate was used in the diet the effect of this alkali was unnoticeable. Perhaps the lime contained

some toxic substance. The rats receiving the lime fared so poorly that only a few of them were put in metabolism cages. In fact, some of them died fairly early. With the exception of those that died too early to show rickets in the x-ray plate, all of the rats in litter 15 developed rickets except rat X. The rats receiving plaster of Paris grew part of the time and their calcium phosphate metabolism was as follows:

This experiment shows that under certain conditions a larger amount of phosphorus in the diet is necessary to prevent rickets than in the experiment of Sherman and Pappenheimer (5). The fact that with 0.2 per cent P a slight rickets developed (which later was cured without change in the diet) indicates that the need for phosphorus in percentage of the diet is greater the greater the growth rate. By placing 150 rats

TABLE 6

Litter 15

Put on diets when 1 month old. Food intake about 6 grams per day

RAT	BODY WEIGHT			GAIN OR LOSS PER RAT PER DAY		X-RAY	RACHITIC INDEX
	50th day	57th day	Gain	Calcium	Phosphorus		
	grams	*grams*	*grams*	*mgm.*	*mgm.*		
VI	43.5	42.5	−1.0	+4.0	+1.0	Rickets	0.7
VII	34.5	30.5	−4.0	+2.0	−0.5	Rickets	1.3
VIII	52.0	54.5	+2.5	+5.0	+1.0	Rickets	0.2
IX	40.0	42.0	+2.0	+6.5	+2.6	Slight rickets	0.0
X	55.0	62.5	+7.0	+7.0	+17.0	Normal (?)	−2.8

on diets differing in P content and growth-promoting substances I found that 0.12 per cent P was sufficient to prevent rickets with loss in weight but more than 0.4 per cent P was required to prevent rickets with normal growth. Spinach (which contains vitamine-A and phosphoric acid) must be given in a very large dose to prevent rickets. In fact, we found that about 40 per cent of spinach in the diet was necessary to prevent rickets in some experiments. From the experiments with litter 12, rats IV and IX were given the additional phosphoric acid in spinach forming 23 per cent of total P. Spinach contains growth-promoting substances and increased growth increases the need for P and rats IV and IX grew faster and had higher rachitic indices. With normal growth in all rats of a series the incorporation of spinach in the diet would not necessitate a higher total P to prevent rickets.

BIBLIOGRAPHY

(1) McCollum and co-workers: Journ. Biol Chem., 1922, i, Proc. Soc. Biol. Chem., p. v.
(2) McCollum, Simmonds, Shipley and Park: Bull. Johns Hopkins Hosp., 1922, xxxiii, 31.
(3) University of Missouri Agric. Research Bull. 28, 1918, table 55.
(4) Jackson: Journ. Exper. Zoöl., 1915, xiv, 99.
(5) Sherman and Pappenheimer: Proc. Soc. Exper. Biol. Med., 1921, xviii, 193.

THE ALKALINE RESERVE OF THE BLOOD OF FISH IN RELATION TO THE ENVIRONMENT[1]

EDWIN B. POWERS

From the University of Nebraska, Lincoln

Received for publication May 15, 1922

Henderson and Haggard (1) state that "The balance between acids and alkalies has come to be recognized as one of the most important of the equilibria of the living body. It is so important in fact that it appears never to be considerably altered except when the organism is practically moribund." This statement is perhaps just as true of fishes as of the land vertebrates. No evidence has been presented to the contrary.

Unless the fish is able by some peculiar mechanism to control the amount of carbon dioxide in the blood (2), the complexity of the relations of oxygen and carbon dioxide and the acid alkaline balance in the blood must be greater than that found in land vertebrates. The author can see no reason why the gas exchange in the respiration of the fish should not follow the same laws of physics and chemistry as in that of higher vertebrates. The only requisites are that the respiratory mechanism be so adjusted that the response to the variations in carbon dioxide and oxygen tensions be more rapid or that the behavior of the fish be such that it will not allow of the fish's moving rapidly between waters of different carbon dioxide or oxygen tensions or both.

The relation of the variation of the carbon dioxide and oxygen tensions in the atmosphere is far less complex than in the hydrosphere. As a general rule in large bodies of water the oxygen tension decreases as the carbon dioxide tension increases. But there is no definite relation between the two. A fish might even experience a decrease or an increase in one or both in passing through a natural outlet or inlet from one water to another. In no case would there necessarily be any relation between the changes of the two tensions.

[1] Studies from the Zoölogical Department, The University of Nebraska, No. 132.

Experiments and observations of the author and other workers have led the author to the opinion that the adjustment of the acid and alkaline equilibria of the blood of certain species of fishes is more rapid than in others. Fish are known (3) to enter water of low oxygen tension (1 cc. or less per l.) and high carbon dioxide tension without any apparent ill effect. The cunner (*Tautogolabrus adspersus* Walbaum) is cosmopolitan around the Woods Hole vicinity and is an all year resident (4). The Pacific Coast herring (*Clupea pallasii* Cuv. and Val.) is known to select in an experimental gradient tank water with a certain definite range of hydrogen ion concentration or perhaps better a certain range of carbon dioxide tension of the sea water (5). The Atlantic Coast herring (*Clupea harengus* L.) disappears from the coast of Sweden simultaneously with the outflow of the bank-water and the inflow of the Baltic Current. Ekman first observed this phenomenon in 1878–1879 (6), (7), (8). Pettersson states, "We have observations which enable us to follow the departure of the herring from one fishing ground to another" (8). Bertram (9), (10) attributes the failure of the herring fishery off Cape Breton to east and north-east storms. Presumably the failure of the fishery was due to a deflection of the ocean current sufficient locally to cause Cape Breton to be bathed by water slightly different in some certain factor or factors which caused the herring to avoid it.

Experiments performed to determine the ability of certain species of fishes to absorb oxygen from the water at low oxygen tension at different hydrogen ion concentrations or carbon dioxide tensions bear out the same views (11). The migratory fishes (*Clupea harengus, C. pallasii, Pomolobus pseudoharengus* Wilson and *Scomber Scombrus* L.) were most affected by either an increase or a decrease in the carbon dioxide of the water while the butter-fish (*Poronotus triacanthus* Peck) was affected less and the cunner (*Tautogolabrus adspersus* Walbaum) least of all. Reaction experiments (12), (5) and observations on the immediate effect of carbon dioxide tension on the rate of oxygen absorption at ordinary oxygen tension (13) add further evidence.

Experiments were planned to determine whether or not a change in carbon dioxide tension of the water would in any case call forth an adjustment in the acid alkaline equilibria in the blood of the fish. The rock-fish (*Sabastodes deani* Starks) was selected as a fish that would perhaps show a rapid adjustment.

Methods and experimental data. Five fish were placed in a live car just off the docks of the laboratory at the Puget Sound Biological Station. Five other fish of as nearly equal sizes as possible were placed in

a tank, $40 \times 11\frac{1}{4} \times 16$ inches deep, of sea water through which carbon dioxide had been passed. After five to seven hours the alkaline reserve of the blood plasma was determined by the colorimetric method described by Marriot (14). The blood was obtained from the caudal artery by severing the tail.

Table 1 gives the results of the tests. The difference in the shade of the color of the dialysate solutions of the five fish kept in the live car was indistinguishable. Size had no effect on the alkaline reserve of the blood plasma. All showed an alkaline reserve as indicated by a pH of 7.65. The five experimental fish showed an alkaline reserve as indicated by a pH of 7.83 to 7.87.

TABLE 1

Showing the relative alkaline reserve of the blood plasma of the rock-fish (Sabastodes deani Starks) as determined by the method described by Marriott (14)

	NUMBER OF FISH	pH OF WATER AT END OF OBSERVATION	OXYGEN PER L. AT END OF OBSERVATION	RELATIVE ALKALINE RESERVE pH	TEMPERATURE
			cc.		°C.
Control	5	7.93	5.92	7.65	11.8
Experiment	5	7.25	1.14	7.83 to 7.87	12.9
			Samples taken from bottom of tank		

Discussion. The data of the experiment show that the akaline reserve of the blood plasma of the rock-fish is changed very rapidly. Since the oxygen content of the water of the experiment was rather low at the close (1.14 cc. per l.) it is not known which of the two factors—low oxygen or high carbon dioxide tension of the sea water—or if both of the two factors were responsible for the bringing about of an increase in the alkaline reserve. The important fact is that there was an increase in the alkaline reserve of the blood plasma of the experimental fish. This opens up another avenue for investigation of the physiological factors controlling the habits of fishes.

It was impossible to obtain sufficient blood from a 9 to 12 gram herring by the methods employed to test its alkaline reserve. However, the herring did not show acclimatization at least to the high carbon dioxide tension of the sea water even after one to two days as determined by the ability to absorb oxygen at a low tension (11). That is, the herring were not able to absorb oxygen at a lower tension after being

in the treated water from one to two days than the herring that had been in the treated water only 1 hour and 23 minutes to 2 hours and 40 minutes.

It is interesting to note at this point that Collip (2) found that the blood of a rock-fish at Protection Gap had a carbon dioxide capacity at atmospheric tension of 8.3 cc. per liter, one at Nanoose Bay 10.2 cc. and one at Departure Bay 11.2 cc. (plasma). The three specimens of herring taken from Departure Bay showed a capacity of 11.2 cc. of the plasma.

CONCLUSIONS

The above facts indicate that certain non-migratory marine fishes are able to change the alkaline reserve of their blood to accommodate the fish to the variations in the carbon dioxide tension or the oxygen tension of the sea water or both.

This ability if present is seemingly less strongly developed in the more migratory fishes such as the Pacific Coast herring.

The author wishes to thank Prof. T. C. Frye, Director of the Puget Sound Biological Station, for rooms, equipment and materials for this work, and for many courtesies during its progress. The author wishes also to thank the Department of Zoölogy, The University of Nebraska, for use of apparatus and material.

BIBLIOGRAPHY

(1) Henderson and Haggard: Journ. Biol. Chem., 1918, xxxiii, 333.
(2) Collip: Journ. Biol. Chem., 1920, xliv, 329.
(3) Birge and Juday: Wisconsin Survey, 1911, Bull. no. xxii.
(4) Sumner, Osburn and Cole: U. S. Bureau of Fisheries, 1911, xxxi, pt. 2, 549.
(5) Powers: Pub. Puget Sound Biol. Sta., Univ. Washington, 1921, iii, 1.
(6) Ekman: Bih. till Göteborg och Bohusläns Hushälen. Sällskaps quartalsskrift. Göteborg, 1880.[2]
(7) Pettersson and Ekman: Kongliga Svenska Vetenskaps-Akademiens Handlingar, 1890–1891, Bd. 24, ii, no. xi, 1.[2]
(8) Pettersson: Scottish Geographic Magazine, 1894, x, 525.
(9) Bertram: Rept. Dept. Marine Fish., 1897, 1896, 40.
(10) Bertram: Rept. Dept. Marine Fish., 1897, 1898, 38.
(11) Powers: Journ. Gen. Physiol, 1922, iv, 305.
(12) Shelford and Powers: Biol. Bull., 1915, xxviii, 315.
(13) Powers: Ecology, 1922.
(14) Marriott: Arch. Int. Med., 1916, xvii, 840.

[2] Not read by the author.